Generation of antimelanoma cytotoxic T lymphocytes from healthy donors after presentation of melanoma-associated antigen-derived epitopes by dendritic cells in vitro.

Bakker AB, Marland G, de Boer AJ, Huijbens RJ, Danen EH, Adema GJ, Figdor CG.

Department of Tumor Immunology, University Hospital Nijmegen St. Radboud, The Netherlands.

MHC class I-restricted CTLs specific for antigens expressed by malignant cells are an important component of immune responses against human cancer. Recently, in melanoma a number of melanocyte differentiation antigens have been identified as potential tumor rejection antigens. In the present study, we show that by applying peptide-loaded dendritic cells, induced by granulocyte-macrophage colony-stimulating factor and interleukin 4 from peripheral blood monocytes of healthy donors, we were able to elicit melanoma-associated antigen-specific CTL in vitro. We demonstrate the induction of CTLs directed against HLA-A2.1 presented epitopes derived from tyrosinase, gp100, and Melan A/MART-1. Apart from lysis of peptide-loaded target cells, these CTLs displayed reactivity with HLA-A2.1+ melanoma tumor cell lines and cultured normal melanocytes endogenously expressing the target antigen. These data indicate that these CTLs recognize naturally processed and presented epitopes and that precursor CTLs against melanocyte differentiation antigens are present in healthy individuals. The ability to generate tumor-specific CTLs in vitro, using granulocyte-macrophage colony-stimulating factor/interleukin 4-induced dendritic cells, illustrates the potential use of this type of antigen-presenting cells for vaccination protocols in human cancer.

PMID: 7585596 [PubMed - indexed for MEDLINE]

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L2 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
2004:392303 Document No. 140:405470 T-cell epitopes of HA-1 minor histocompatibility antigen for immunotherapy. Goulmy, Elsa A. J. M.; Hunt, Donald F.; Engelhard, Victor H. (Neth.). U.S. Pat. Appl. Publ. US 2004092446 A1 20040513, 69 pp., Cont.-in-part of U.S. Ser. No. 489,760. (English). CODEN: USXXCO. APPLICATION: US 2003-623176 20030718. PRIORITY: EP 1997-202303 19970723; WO 1998-NL424 19980723; US 2000-489760 20000121.

AB The authors disclose peptides constituting T-cell epitopes of the minor histocompatibility antigen, HA-1. The authors demonstrate that HA-1 is associated with graft vs. host disease, is expressed by non-hematopoietic tumor cells, and can be recognized by **HA-1 cytotoxic T cells.** The peptides and their derivs. find many uses, for instance in bone marrow transplantation, organ transplantation and in treatment of leukemia and non-hematopoietic tumors. The peptide and/or its derivs. can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits.

L2 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
2003:450776 Document No. 139:21025 Minor histocompatibility antigen HA-1: target antigen for immunotherapy of tumors. Goulmy, Elsa Afra Julia Maria (Academisch Ziekenhuis Leiden, Neth.). Eur. Pat. Appl. EP 1317927 A1 20030611, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-204704 20011205.

AB Allogeneic stem cell transplants (SCT) can induce curative Graft vs. Tumor (GvT) reactivities in patients with hematol. malignancies. The GvT reaction is mainly mediated by allo immune donor T-cells specific for polymorphic minor Histocompatibility antigens (mHags). Among these, the mHag HA-1 was restricted to the hematopoietic system. Here the authors report the expression of HA-1 by non-hematopoietic tumor cells. While absent in normal epithelial cells, tumor cells, tumor cell lines particularly from epithelial origin, also express HA-1 and are recognized by **HA-1 cytotoxic T cells**. The invention provides, among others, means and methods for HA-1 specific immunotherapy for HA-1 pos. patients with non-hematopoietic tumor cells.

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L12  ANSWER 1 OF 26  CAPLUS  COPYRIGHT 2004 ACS on STN
2004:392303   Document No. 140:405470  T-cell epitopes of HA-
     1 minor histocompatibility antigen for immunotherapy.  Goulmy,
     Elsa A. J. M.; Hunt, Donald F.; Engelhard, Victor H. (Neth.).  U.S. Pat.
     Appl. Publ. US 2004092446 A1 20040513, 69 pp., Cont.-in-part of U.S. Ser.
     No. 489,760.  (English).  CODEN: USXXCO.  APPLICATION: US 2003-623176
     20030718.  PRIORITY: EP 1997-202303 19970723; WO 1998-NL424 19980723; US
     2000-489760 20000121.
AB   The authors disclose peptides constituting T-cell epitopes of
     the minor histocompatibility antigen, HA-1.  The
     authors demonstrate that HA-1 is associated with graft
     vs. host disease, is expressed by non-hematopoietic tumor cells, and can
     be recognized by HA-1 cytotoxic T
     cells.  The peptides and their derivs. find many uses,
     for instance in bone marrow transplantation, organ transplantation and in
     treatment of leukemia and non-hematopoietic tumors.  The peptide
     and/or its derivs. can be incorporated in vaccines, in pharmaceutical
     formulations and they can be used in diagnostic test kits.

L12  ANSWER 2 OF 26     MEDLINE on STN
2004085970.    PubMed ID: 14592836.   Pregnancy induces minor
     histocompatibility antigen-specific cytotoxic T
     cells: implications for stem cell transplantation and
     immunotherapy. Verdijk Rob M; Kloosterman Antoinette; Pool Jos; van de
     Keur Maarten; Naipal Albert M I H; van Halteren Astrid G S; Brand Anneke;
     Mutis Tuna; Goulmy Els. (Department of Immunohematology and Blood
     Transfusion, Leiden University Medical Center, Leiden, The Netherlands. )
     Blood, (2004 Mar 1) 103 (5) 1961-4.  Journal code: 7603509. ISSN:
     0006-4971. Pub. country: United States. Language: English.
AB   Recipients of HLA-identical stem cell transplants have a poorer transplant
     outcome if the donor is female rather than male.  We analyzed whether
     pregnancy primes for minor histocompatibility (H) antigens.  Peripheral
     blood mononuclear cells (PBMCs) from healthy multiparous female blood
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donors were depleted for CD4+, CD14+, CD16+, and CD19+ cells, stained with minor H antigen-specific HLA-A2 tetramers, sorted by fluorescence-activated cell sorting, and tested for cytotoxic activity. Minor H antigens HY-, **HA-1**-, and HA-2-specific **cytotoxic T cells** (CD8+, CD45RA-) were present in PBMCs from 4 of 7 female donors up to 22 years after the last delivery. Interestingly, in 2 of the 4 cases microchimerism of the putative immunizing minor H antigen was observed. Thus, pregnancy can lead to alloimmune responses against the infant's paternal minor H antigens. The minor H antigen immunization status of female donors raises important questions for the clinical practice of stem cell transplantation.

L12 ANSWER 3 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN

2004:571330 Document No. 141:258993 Artificial antigen-presenting constructs efficiently stimulate minor histocompatibility antigen-specific cytotoxic T lymphocytes. Oosten, Liesbeth E. M.; Blokland, Els; Van Halteren, Astrid G. S.; Curtsinger, Julie; Mescher, Matthew F.; Falkenburg, J. H. Frederik; Mutis, Tuna; Goulmy, Els (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Neth.). Blood, 104(1), 224-226 (English) 2004. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.

AB Cytotoxic T lymphocytes (CTLs) specific for hematopoietic-restricted minor histocompatibility antigens (mHags) are important reagents for adoptive immunotherapy of relapsed leukemia after allogeneic stem cell transplantation. However, expansion of these CTLs to therapeutic nos. is often hampered by the limited supply of antigen-presenting cells (APCs). Therefore, the authors evaluated whether cell-sized latex beads coated with HLA/mHag complexes HLA-A2/**HA-1** or HLA-A2/HA-2 and recombinant CD80 and CD54 mols. can replace professional APCs. The artificial antigen-presenting constructs (aAPCs) effectively stimulated **HA-1**-and HA-2-specific CTL clones as shown by ligand-specific expansion, cytokine production, and maintenance of cytotoxic activity, without alteration of CTL phenotype. Furthermore, **HA-1**-specific polyclonal CTL lines were enriched as efficiently by aAPCs as by autologous **HA-1 peptide**-pulsed dendritic cells. Thus, aAPCs coated with HLA/mHag complexes, CD80, and CD54 may serve as tools for in vitro enrichment of immunotherapeutic mHag-specific CTL lines.

L12 ANSWER 4 OF 26 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2004:189798 The Genuine Article (R) Number: 775KG. Minor histocompatibility antigens: Allo target molecules for tumor-specific immunotherapy. Goulmy E (Reprint). Leiden Univ, Med Ctr, Dept Immunohaematol & Bloodtransfus, Post Box 9600, NL-2300 RC Leiden, Netherlands (Reprint); Leiden Univ, Med Ctr, Dept Immunohaematol & Bloodtransfus, NL-2300 RC Leiden, Netherlands. CANCER JOURNAL (JAN-FEB 2004) Vol. 10, No. 1, pp. 1-7. Publisher: JONES AND BARTLETT PUBLISHERS. 40 TALL PINE DR, SUDBURY, MA 01776 USA. ISSN: 1528-9117. Pub. country: Netherlands. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Minor histocompatibility antigens have to be considered as key molecules in the stem cell-based immunotherapy of malignancies. Allogeneic stem cell transplantation (SCT) is a well-established and effective therapy for advanced hematologic malignancies. The apparent powerful graft-versus-leukemia effect of SCT led clinicians to apply SCT for the treatment of metastatic solid tumors. The SCT-based graft-versus-tumor reaction in the allogeneic human leukocyte antigen-matched SCT setting is mediated by allo-immune effector cells directed against tumor-related target antigens. The target molecules involved in the allo-immune graft-versus-tumor reaction are tumor-specific antigens, tumor-associated antigens, and tissue- and cell-specific minor histocompatibility antigens. The power of the minor histocompatibility antigens in the human leukocyte antigen-identical, stem cell-based immunotherapy for malignancies is their "allo-ness." As opposed to tumor-associated self antigens, the complexes of MHC and allo-target **peptide** are likely to be more

immunogeneic than the major histocompatibility complex and self-target **peptide** complexes. Moreover, minor histocompatibility allo-antigens are not subject to self tolerance. Earlier minor histocompatibility antigens were seen as alien entities, disturbing the success of the so ideally matched organ and SCT donor-recipient combinations. To date, minor histocompatibility antigens can be set in the favorable light of useful tools for immunotherapy for cancer. The first clinical application of the hematopoietic minor histocompatibility antigens **HA-1** and HA2 is currently being explored in a stem cell-based setting for hematologic malignancies. Because **HA -1** is also expressed on carcinoma cells, a stem cell-based vaccination trial for patients with metastatic breast or renal cancer is about to start as well. The immunotherapeutic potential of minor histocompatibility antigens demands serious searches for new minor histocompatibility antigens and analyses of their phenotype frequency, tissue distribution, and functional membrane expression. The minor histocompatibility antigens meeting the prerequisites for specific immunotherapy for malignancies, such as membrane expression and tissue and/or cell specificity, may offer the curative tools for stem cell-based immunotherapy for various hematologic and nonhematologic malignancies.

L12 ANSWER 5 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN
2003:64824 Document No. 139:5250 Competition-based cellular **peptide** binding assays for 13 prevalent HLA class I alleles using fluorescein-labeled synthetic **peptides.** Kessler, Jan H.; Mommaas, Bregje; Mutis, Tuna; Huijbers, Ivo; Vissers, Debby; Benckhuijsen, Willemien E.; Schreuder, Geziena M. Th.; Offringa, Rienk; Goulmy, Els; Melief, Cornelis J. M.; van der Burg, Sjoerd H.; Drijfhout, Jan W. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, 2300 RC, Neth.). Human Immunology, 64(2), 245-255 (English) 2003. CODEN: HUIMDQ. ISSN: 0198-8859. Publisher: Elsevier Science Inc..

AB We report the development, validation, and application of competition-based **peptide** binding assays for 13 prevalent human leukocyte antigen (HLA) class I alleles. The assays are based on **peptide** binding to HLA mols. on living cells carrying the particular allele. Competition for binding between the test **peptide** of interest and a fluorescein-labeled HLA class I binding **peptide** is used as read out. The use of cell membrane-bound HLA class I mols. circumvents the need for laborious biochem. purification of these mols. in soluble form. Previously, we have applied this principle for HLA-A2 and HLA-A3. We now describe the assays for HLA-A1, HLA-A11, HLA-A24, HLA-A68, HLA-B7, HLA-B8, HLA-B14, HLA-B35, HLA-B60, HLA-B61, and HLA-B62. Together with HLA-A2 and HLA-A3, these alleles cover more than 95% of the Caucasian population. Several allele-specific parameters were determined for each assay. Using these assays, we identified novel HLA class I high-affinity binding **peptides** from HIVpol, p53, PRAME, and minor histocompatibility antigen **HA-1.** Thus, these convenient and accurate **peptide**-binding assays will be useful for the identification of putative cytotoxic T lymphocyte epitopes presented on a diverse array of HLA class I mols.

L12 ANSWER 6 OF 26 MEDLINE on STN DUPLICATE 1
2003277304. PubMed ID: 12804533. Potential limitations in using minor histocompatibility antigen-specific **cytotoxic T cells** for targeting solid tumor cells. Miyazaki Mikinori; Akatsuka Yoshiki; Nishida Tetsuya; Fujii Nobuharu; Hiraki Akio; Ikeda Kazuma; Tsujimura Kunio; Kuzushima Kiyotaka; Morishima Yasuo; Sato Shigeki; Ueda Ryuzo; Takahashi Toshitada. (Division of Immunology, Aichi Cancer Center Research Institute, Nagoya, Japan.) Clinical immunology (Orlando, Fla.), (2003 Jun) 107 (3) 198-201. Journal code: 100883537. ISSN: 1521-6616. Pub. country: United States. Language: English.

AB We have shown previously that KIAA0223, a gene encoding a minor histocompatibility antigen, **HA-1**, whose expression was believed to be restricted to the hematopoietic cells, is aberrantly

expressed in some solid tumor cell lines. However, its significance in tumor immunity needs to be determined. Cytotoxic activity of **HA-1**(H)-specific cytotoxic T lymphocytes (CTLs) was assessed against solid tumor cell lines expressing KIAA0223 using (51)Cr release assays. Five of seven cell lines were lysed when HLA-A*0201 was adequately expressed. One of the two CTL-resistant cell lines became susceptible after treatment with IFN-gamma and TNF-alpha, while the other was lysed only after pulsing with **HA-1**(H) **peptide**. In most cell lines tested, **HA-1**(H) **peptide** was properly generated and presented for recognition by the CTL. However, impaired antigen processing and presentation observed in this study may result in escape from CTL recognition in vivo, as well as in vitro, as observed in this study.

L12 ANSWER 7 OF 26 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2004:59211 Document No.: PREV200400054629. Minor histocompatibility antigens **HA-1** and HPA-5 polymorphism in HLA identical related bone marrow transplantation. Spyropoulou-Vlachou, Maria [Reprint Author]; Kotzampasaki, Valia [Reprint Author]; Papadimitropoulos, Miltiadis [Reprint Author]; Stavropoulos-Giokas, Catherine [Reprint Author]. Immunology Dp.-National Tissue Typing Lab., National Hospital of Athens G.Gennimatas, Athens, Greece. Human Immunology, (2003) Vol. 64, No. Supplement 1, pp. S57. print.
Meeting Info.: 29th Annual Meeting of the American Society for Histocompatibility and Immunogenetics. Miami Beach, FL, USA. October 28-November 01, 2003. American Society for Histocompatibility and Immunogenetics.
CODEN: HUIMDQ. ISSN: 0198-8859. Language: English.

L12 ANSWER 8 OF 26 MEDLINE on STN DUPLICATE 2

2002459659. PubMed ID: 12218130. Identification of a novel HLA-B60-restricted T cell epitope of the minor histocompatibility antigen **HA-1** locus. Mommaas Bregje; Kamp Janine; Drijfhout Jan-Wouter; Beekman Nico; Ossendorp Ferry; Van Veelen Peter; Den Haan Joke; Goulmy Els; Mutis Tuna. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.) Journal of immunology (Baltimore, Md. : 1950), (2002 Sep 15) 169 (6) 3131-6. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The polymorphic minor histocompatibility Ag **HA-1** locus encodes two **peptides**, **HA-1**(H) and **HA-1**(R), with a single amino acid difference. Whereas the immunogenicity of the **HA-1**(R) allele has not yet been shown, the nonameric **HA-1**(H) **peptide** induces HLA-A2-restricted **cytotoxic T cells** in vivo and in vitro. It is not known whether the mHag **HA-1**(H) or **HA-1**(R) associates with other HLA class I molecules. Therefore, the polymorphic regions of both **HA-1** alleles were analyzed to identify HLA class I binding **peptides** that are properly processed by proteasomal degradation. **Peptide** binding analyses were performed for all nonameric **HA-1**(H/R) **peptides** for binding to nine HLA class I molecules with >10% prevalence in the Caucasian population and for seven nonameric/decameric **HA-1**(H/R) **peptides** predicted to bind to HLA-A3, -B14, and -B60. Only the nonameric KECVL(H)/(R)DDL and decameric KECVL(H)/(R)DDLL **peptides** showed strong and stable binding to HLA-B60. In vitro digestion of 29-aa-long **HA-1 peptides** by purified 20S proteasomes revealed proper cleavage at the COOH termini of both HLA-B60 binding **HA-1**(H) and **HA-1**(R) **peptides**. In subsequent analyses, dendritic cells pulsed with the nonameric **HA-1**(R) **peptide** did not induce CTLs that recognize the natural HLA-B60/**HA-1**(R) ligand. In contrast, dendritic cells pulsed with the nonameric **HA-1**

(H) **peptide** induced IFN-gamma-secreting T cells specific for the natural HLA-B60/**HA-1**(H) ligand in three HLA-B60(+) **HA-1**(RR) individuals, demonstrating the immunogenicity of the HLA-B60/**HA-1**(H) ligand. In conclusion, this study shows a novel HLA-B60-restricted T cell epitope of the minor histocompatibility Ag **HA-1** locus.

L12 ANSWER 9 OF 26 MEDLINE on STN DUPLICATE 3
2002080614. PubMed ID: 11807003. General T-cell receptor antagonists to immunomodulate HLA-A2-restricted minor histocompatibility antigen **HA-1**-specific T-cell responses. den Haan Joke M M; Mutis Tuna; Blokland Els; IJzerman Ad P; Goulmy Els. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden University, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.) Blood, (2002 Feb 1) 99 (3) 985-92. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB T-cell receptors (TCRs) of a series of minor histocompatibility antigen (mHag) **HA-1**-specific **cytotoxic T-cell** (CTL) clones isolated from 3 unrelated patients have been shown to use the same BV6S4A2 segment with conserved amino acids in the CDR3Vbeta region. This suggests that different **HA-1**-specific TCRs interact similarly to the **HA-1** antigen presented by the HLA-A2 molecule. The mHag **HA-1** forms an immunogenic complex with HLA-A2 and induces strong alloimmune responses after stem cell transplantation (SCT). It was questioned, therefore, whether clonal and polyclonal **HA-1**-specific CTL responses can be antagonized by a single TCR antagonistic **peptide**. Functional analysis and molecular modeling of single and double amino acid substitutions of TCR contact residues, adjacent residues, and HLA-A2 binding residues resulted in 4 **peptides** with high affinity for HLA-A2 and with the capacity to inhibit the lysis of endogenously **HA-1**-expressing EBV-BLCL by 3 different **HA-1**-specific CTL clones. These **peptides** also efficiently antagonized **HA-1**-specific polyclonal CTL lines derived from 3 patients and significantly reduced the number of interferon-gamma-producing **HA-1**-specific CTL of a patient with graft-versus-host disease after **HA-1**-mismatched SCT. These data show that general TCR antagonists can be developed that inhibit HLA-A2-restricted **HA-1**-specific CTL responses on the clonal and the polyclonal level and that TCR antagonists may modulate the immunodominant mHag **HA-1** responses.

L12 ANSWER 10 OF 26 MEDLINE on STN DUPLICATE 4
2002348185. PubMed ID: 12091347. Generation of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** restricted by nonself HLA molecules: a potential strategy to treat relapsed leukemia after HLA-mismatched stem cell transplantation. Mutis Tuna; Blokland Els; Kester Michel; Schrama Ellen; Goulmy Els. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands.. t.mutis@lumc.nl) . Blood, (2002 Jul 15) 100 (2) 547-52. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Successful stem cell transplantation (SCT) across HLA barriers can be performed with cord blood, megadoses of stem cells, or with nonmyeloablative conditioning strategies. Because the HLA-mismatched transplants are often T-cell depleted, leukemia relapse rates are high. Treatment of relapsed leukemia after HLA-mismatched SCT is difficult. A novel potential strategy to treat relapsed leukemia after HLA-mismatched SCT is the use of patients' mismatched HLA molecules as antigen-presenting molecules to generate hematopoietic system-specific **cytotoxic T cells** (CTLs) from the stem cell donor. Adoptive transfer of these hematopoietic system-specific CTLs that are restricted by nonself HLA molecules may eliminate leukemia without affecting the patient's nonhematopoietic cells or donor hematopoietic cells. We

investigated the feasibility of this strategy using the hematopoietic system-specific minor histocompatibility antigen **HA-1**, which is known to induce HLA-A2-restricted CTLs. HLA-A2(-) peripheral blood mononuclear cells were stimulated with HLA-A2(+) T2 cells pulsed with synthetic **HA-1 peptide** or with dendritic cells transduced with the **HA-1** cDNA. Tetrameric HLA-A2/**HA-1 peptide** complexes were used to monitor and enrich **HA-1**-specific CTLs. In the alloreactive cultures, **HA-1**-specific CTLs were enriched up to 7% by 3 rounds of antigen-specific stimulations and up to 87% by fluorescence-activated cell sorting of tetramer-positive T cells. The **HA-1**-specific CTLs showed specific lysis of the relevant target cells, including leukemic cells. Because the polyclonal CTL cultures also contained natural killer cells and allo-HLA-A2-specific CTLs, CTL clones were generated that showed the expected **HA-1** specificity only. Thus, **HA-1**-specific CTLs restricted by nonself HLA-A2 molecules can be generated in an HLA-A2-mismatched setting.

L12 ANSWER 11 OF 26 MEDLINE on STN DUPLICATE 5

2002472540. PubMed ID: 12234166. Efficient induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T-cells** using dendritic cells retrovirally transduced with **HA-1**-coding cDNA. Mutis Tuna; Ghoreschi Kamran; Schrama Ellen; Kamp Janine; Heemskerk Mirjam; Falkenburg J H Frederik; Wilke Martina; Goulmy Els. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands.. t.mutis@lumc.nl) . Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, (2002) 8 (8) 412-9. Journal code: 9600628. ISSN: 1083-8791. Pub. country: United States. Language: English.

AB **Cytotoxic T-cells** (CTLs) specific for the hematopoietic system-restricted minor histocompatibility antigen (mHag) **HA-1** efficiently lyse **HA-1**-positive leukemic cells without affecting nonhematopoietic cells. **HA-1**-specific CTLs are thus potential tools for adoptive immunotherapy of relapsed leukemia after HLA-matched-**HA-1**-mismatched stem cell transplantation (SCT). In vitro generation of **HA-1**-specific CTLs from SC donors is possible using dendritic cells (DCs) pulsed with synthetic **HA-1 peptide** as stimulator cells. However, this approach requires at least 6 weeks of in vitro culturing under GMP (good manufacturing practice) conditions. Our data show that in vitro induction of **HA-1**-specific CTLs is more rapid with the use of DCs that are retrovirally transduced with the **HA-1** complementary DNA. Retrovirally transduced DCs showed functional and long-term stable expression of the **HA-1** CTL epitope in primary CTL cultures. In 4 SC donors, **HA-1**-transduced DCs induced **HA-1**-specific CTLs in 14 to 21 days. The in vitro-generated CTL lines contained 6% to 9% T-cells that stained brightly with tetrameric HLA-A2/**HA-1 peptide** complexes (**HA-1**(A2) tetramer) and showed significant lysis of **HA-1**+ leukemic cells. The CTL induction procedure using **peptide**-pulsed DCs was less effective and required 28 to 35 days of T-cell culture. Thus, sustained presentation of mHag **HA-1** by retrovirally transduced DCs facilitates the in vitro induction of **HA-1**-specific CTLs.

L12 ANSWER 12 OF 26 MEDLINE on STN DUPLICATE 6

2002194878. PubMed ID: 11927949. In situ dissection of the graft-versus-host activities of **cytotoxic T cells** specific for minor histocompatibility antigens. Dickinson Anne M; Wang Xiao-Nong; Sviland Lisbet; Vyth-Dreese Florry A; Jackson Graham H; Schumacher Ton N M; Haanen John B A G; Mutis Tuna; Goulmy Els. (University Department of Haematology, Royal Victoria Infirmary,

University of Newcastle, Newcastle upon Tyne, UK.) Nature medicine, (2002 Apr) 8 (4) 410-4. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB Minor histocompatibility antigens (mHags) are immunogenic **peptides** from polymorphic cellular proteins that induce strong T-cell responses after human leukocyte antigen (HLA)-matched, mHag-mismatched stem-cell transplantation. mHags with broad or limited tissue expression are target antigens for graft-versus-host (GvH) and graft-versus-leukemia (GvL) reactivities. Separation of these activities is crucial for adoptive immunotherapy of leukemia without GvH disease. Therefore, using a skin-explant assay we investigated the in situ activities of cytotoxic T lymphocytes (CTLs) specific for the ubiquitously expressed mHag H-Y and for the hematopoietic-restricted mHags **HA-1** and HA-2. H-Y-specific CTLs, visualized by tetrameric HLA-mHag **peptide** complexes, infiltrated male skin sections within 24 hours, induced severe GvH reactions of grade III-IV and produced high levels of IFN-gamma. In contrast, CTLs specific for the hematopoietic system-specific mHags **HA-1** and HA-2 induced no or low GvH reactions above background and produced little or no interferon-gamma, unless the skin sections were preincubated with **HA-1**/HA-2 synthetic **peptides.** These results provide the first in situ dissection of GvH effects by mHag-specific CTLs and show that ubiquitously expressed mHags are the prime targets of GvH disease.

L12 ANSWER 13 OF 26 MEDLINE on STN DUPLICATE 7

2002425399. PubMed ID: 12163564. The hematopoietic system-specific minor histocompatibility antigen **HA-1** shows aberrant expression in epithelial cancer cells. Klein Christoph A; Wilke Martina; Pool Jos; Vermeulen Corine; Blokland Els; Burghart Elke; Krostina Sabine; Wendler Nicole; Passlick Bernward; Riethmueller Gert; Goulmy Els. (Department of Immunology, Klinikum Innenstadt, Ludwig-Maximilians University, 80336 Munich, Germany.. E.A.J.M.Goulmy@lumc.nl) . Journal of experimental medicine, (2002 Aug 5) 196 (3) 359-68. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Allogeneic stem cell transplantation (SCT) can induce curative graft-versus-tumor reactions in patients with hematological malignancies and solid tumors. The graft-versus-tumor reaction after human histocompatibility leukocyte antigen (HLA)-identical SCT is mediated by alloimmune donor T cells specific for polymorphic minor histocompatibility antigens (mHags). Among these, the mHag **HA-1** was found to be restricted to the hematopoietic system. Here, we report on the **HA-1** ribonucleic acid expression by microdissected carcinoma tissues and by single disseminated tumor cells isolated from patients with various epithelial tumors. The **HA-1** **peptide** is molecularly defined, as it forms an immunogenic **peptide** ligand with HLA-A2 on the cell membrane of carcinoma cell lines. **HA-1**-specific **cytotoxic T cells** lyse epithelial tumor cell lines in vitro, whereas normal epithelial cells are not recognized. Thus, **HA-1** -specific immunotherapy combined with HLA-identical allogeneic SCT may now be feasible for patients with **HA-1**(+) carcinomas.

L12 ANSWER 14 OF 26 MEDLINE on STN DUPLICATE 8

2003015783. PubMed ID: 12522448. Exclusive TCRVbeta chain usage of ex vivo generated minor Histocompatibility antigen **HA-1** specific **cytotoxic T cells**: implications for monitoring of immunotherapy of leukemia by TCRBV spectratyping. Verdijk Rob M; Mutis Tuna; Wilke Martina; Pool Jos; Schrama Ellen; Brand Anneke; Goulmy Els. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.. rmverdijk@lumc.nl) . hematology journal : official journal of the European Haematology Association / EHA, (2002) 3 (6) 271-5. Journal code: 100965523. ISSN: 1466-4860. Pub. country: England: United Kingdom. Language: English.

AB Tissue expression of minor Histocompatibility antigens **HA-1** and HA-2 is limited to the hematopoietic system. Therefore, ex

vivo generated **HA-1**/HA-2 specific cytotoxic T lymphocytes (CTLs) can be applied for adoptive immunotherapy of relapsed leukemia after HLA-matched **HA-1**/HA-2 mismatched stem cell transplantation. Here we used T cell receptor beta variable chain (TCRBV) spectratyping and/or TCRBV sequencing to monitor the specific TCR usage in eleven **HA-1**/HA-2 CTLs that were induced ex vivo with **peptide** pulsed dendritic cells. The HA-2 induced CTLs used different TCRBV. In contrast, the development of **HA-1** specific CTLs coincided with prominent skewing of TCRBV7 spectratypes. Sequencing of the TCRBV7 specific PCR products used by these ex vivo generated **HA-1** CTLs revealed the exclusive usage of TCRBV7-9*03, identical to the TCRBV used by **HA-1** specific CTLs induced in vivo after stem cell transplantation. Thus, monitoring of immunotherapy with **HA-1** specific CTLs is now also feasible by TCRBV spectratyping.

L12 ANSWER 15 OF 26 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2003:357430 Document No.: PREV200300357430. Non-Availability of Clinical Grade Reagents Prohibits the Clinical Application of In Vitro Cultured **Peptide**-Specific Cytotoxic T Lymphocytes(CTL). Marijt, W. A. F. [Reprint Author]; Bergen, C. A. M. van [Reprint Author]; Hoorn, M. A. W. G. van [Reprint Author]; Muijsenberg, J. W. van den [Reprint Author]; Willemze, R. [Reprint Author]; Falkenburg, J. H. F. [Reprint Author]. Hematology, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 3295. print. Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB CTL with specificity for the hematopoiesis-associated mHag **HA-1** and/or HA-2 can be generated from healthy stem cell donors using mature dendritic cells (DC) loaded with **HA-1** or -2 **peptide** as stimulator cells. Administration of such CTL instead of unmanipulated DLI to patients with relapsed leukemia after alloSCT may induce a GVL effect with little or no GVHD. Clinical grade DC can be generated from CD14+ or CD34+ cells cultured in the presence of GM-CSF, SCF, IL-4 and/or TNF. However, maturation of CD14 or CD34 derived DC is essential for efficient induction of mHag specific CTL from unprimed individuals. By addition of various maturation factors such as alpha-IFN, TNF, polyI:C, or CPG to DC during the last 2 days of culture, partial maturation can be obtained as shown by upregulation of CD40, and CD86, but often only modest or no upregulation of CD80 and CD83 is found. Unfortunately, we were not able to frequently induce a primary response and generate sufficient numbers of mHag specific CTL using partially maturated **peptide** loaded DC as stimulator cells. In contrast, when naive T cells were stimulated with **HA-1** loaded DC that were fully maturated by addition of CD40-L transfected mouse fibroblasts (CD40-L cells) resulting in high expression of CD80 and CD83 molecules on a higher number of DC (mean fluorescence intensity 871+-461 and 229+-35 in the presence of CD40-L cells, versus 162+-39 and 47+-20, respectively, without CD40-L cells) large numbers of highly **cytotoxic T cells** could be generated consisting of up to 45% **HA-1** specific T cells as demonstrated by staining with HLA-A2/**HA-1** tetramers. Cytotoxicity assays showed 55% lysis of PHA blasts loaded with **HA-1 peptide**, 30% lysis of PHA blasts expressing endogenous **HA-1 peptide** and no lysis of **HA-1** negative PHA blasts (E:T ratio of 1:1). Production of IL-12 by these mature DC was high (1988+-883 pg/mL) emphasizing their capacity to induce a primary immune response. However, CD40-L cells are not approved for clinical use. Therefore, we attempted to use alternative maturation stimuli that are approved for clinical use such as DKTP (diphtheria, whooping cough, tetanus toxoid, and polio) vaccine, or tetanus toxoid (TT) vaccine. Addition of DKTP or TT to DC cultures

induced 30-40% less upregulation of CD80- and CD83 molecules compared to maturation with CD40-L cells. Furthermore, production of IL-12 was much lower (6+-10 pg/mL) and IL-10 production was relatively high. Using DKTP maturated, **HA-1** loaded DC only limited numbers of specific CTL could be induced. An alternative to the use of CD40-L cells might be the addition of effective amounts clinical grade IL-12 to the CTL cultures to bypass the lack of fully matured DC. However, clinical grade IL-12 has not been made available. In conclusion, we show that it is feasible to fully maturate CD14 and CD34 derived DC with CD40-L cells as measured by the high upregulation of CD80 and CD83 expression and substantial production of IL-12 resulting in the generation of **HA -1** tetramer+ CTL and lysis of **HA-1**+ target cells. Alternative maturational stimuli, such as DKTP or TT, which are approved for clinical use could not replace CD40-L cells. Currently, one of the major obstacles for efficient clinical application of antigen specific cellular immunotherapy to treat patients with relapsed leukemia after alloSCT is the unavailability of clinical grade reagents.

L12 ANSWER 16 OF 26 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2002:220339 Document No.: PREV200200220339. Minor histocompatibility antigen specific CTLs and in situ skin graft versus host reactions. Wang, Xiao-Nong [Reprint author]; Dickinson, Anne M. [Reprint author]; Sviland, Lisbet; Vyth-Dreese, F. A.; Jackson, Graham H. [Reprint author]; Schumacher, Ton N. M.; Haanen, John B. A. G.; Mutis, Tuna; Goulmy, Els. University Department of Haematology, Royal Victoria Infirmary, Newcastle Upon Tyne, UK. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 649a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Minor Histocompatibility antigens (mHags) are immunogenic **peptides** from polymorphic cellular proteins that induce strong T cell responses after HLA matched mHag mismatched stem cell transplantation (SCT). mHags with broad or limited tissue expression are target antigens for Graft versus Host (GvH) or Graft versus Leukemia (GvL) reactivities, respectively. Dissection between these activities is crucial for adoptive immunotherapy of leukemia without Graft versus Host Disease (GvHD). Therefore, we investigated the in situ behaviour of **cytotoxic T cells** (CTLs) specific for the ubiquitously expressed mHag H-Y and for the hematopoietic restricted mHags **HA-1** and HA-2 in a skin explant assay. H-Y specific CTLs, visualized by tetrameric HLA/mHag **peptide** complexes, infiltrated male skin sections within 24 hours, induced severe GvH reactions of grade III-IV and produced high levels of IFN-gamma. In striking contrast, CTLs specific for the hematopoietic system specific mHags **HA-1** and HA-2 induced no or low GvH reactions above background and produced no or little IFN-gamma, unless the skin sections were preincubated with **HA-1**/HA-2 synthetic **peptides.** These results provide the first in situ dissection of GvH and GvL and prove that ubiquitously expressed mHags are the prime targets of GvHD. The results also underline that CTLs specific for the hematopoietic system specific mHags **HA-1** and HA-2 can be safely applied for the treatment of relapsed leukemia.

L12 ANSWER 17 OF 26 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:312013 Document No.: PREV200100312013. Efficient in vitro induction of minor histocompatibility antigen **HA-1** specific cytotoxic T-lymphocytes using dendritic cells retrovirally transduced with **HA-1** coding gene segment. Mutis, Tuna [Reprint author]; Wilke, Martina [Reprint author]; Ghoreschi, Kamran; Schrama, Ellen [Reprint author]; Kamp, Janine [Reprint author]; Heemskerk, Mirjam; Falkenburg, J. H. Frederik; Goulmy, Els [Reprint author]. Dept. of

Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 582a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB The minor histocompatibility antigen (mHag) **HA-1** is a hematopoietic system specific polymorphic antigen that can be recognized by **cytotoxic T cells** (CTLs) in the context of HLA-A2. **HA-1** specific CTLs exhibit strong anti-leukemia reactivity by lysing **HA-1** positive leukemic cells and their clonogenic precursors without affecting non-hematopoietic cells. Adoptive transfer of in vitro generated **HA-1** specific CTLs into **HA-1** positive patients with relapsed leukemia may therefore be curative with a low risk of graft versus host disease (GvHD). We have recently shown the feasibility of in vitro generation of **HA-1** specific CTLs from **HA-1** negative individuals using dendritic cells (DCs) pulsed with synthetic **HA-1 peptide** . However, under GMP conditions, **HA-1** CTLs can not be generated from some donors using **peptide** pulsed DCs. We therefore investigated whether generation of **HA-1** specific CTLs is more effective using DCs that are retrovirally transduced to express the **HA-1** antigen. The 312 base pair gene segment coding for the **HA-1** CTL epitope was cloned into the retroviral vector LZRS. This vector was transduced into several cell lines including CD34+ DC progenitors with 10-40% efficiency. All retrovirally transduced cells showed stable and functional expression of the **HA-1** CTL epitope. CD34+ DC progenitors differentiated normally into immature DCs within 10-12 days and induced strong in vitro **HA-1** specific CTL responses in four out of six **HA-1** negative healthy unprimed individuals. The CTL lines contained 6-10% **HA-1** specific CTLs as determined by HLA-A2/**HA-1 peptide** tetramers. The induction of **HA-1** specific CTLs by retrovirally transduced DCs required only one or two rounds of restimulation whereas CTL induction by **peptide** pulsed DCs required three to five rounds of restimulations. During the CTL induction, the retrovirally transduced DCs were detected at least seven days in the cultures and retained their immature phenotype. Our results demonstrate that retrovirally transduced immature DCs effectively induce **HA-1** specific CTL responses through their continuous presentation of the **HA-1** T cell epitope to unprimed T cell precursors.

L12 ANSWER 18 OF 26 MEDLINE on STN

2002187510. PubMed ID: 11920221. HLA class I-minor histocompatibility antigen tetramers select **cytotoxic T cells** with high avidity to the natural ligand. Gillespie G; Mutis T; Schrama E; Kamp J; Esendam B; Falkenburg J F; Goulmy E; Moss P. (Molecular Immunology Group, John Radcliffe Hospital, Oxford, UK.) hematology journal : official journal of the European Haematology Association / EHA, (2000) 1 (6) 403-10. Journal code: 100965523. ISSN: 1466-4860. Pub. country: England: United Kingdom. Language: English.

AB INTRODUCTION: **Cytotoxic T cells** specific for the hematopoietic system-restricted minor histocompatibility antigens **HA-1** and HA-2 are potential tools for the treatment of relapsed leukemia after minor histocompatibility antigen mismatched bone marrow transplantation. **HA-1**/HA-2-specific **cytotoxic T cells** with strong cytotoxic activity against **HA-1**/HA-2 positive target cells can be generated in vitro using **HA-1** and HA-2 **peptide**-pulsed dendritic cells as antigen presenting cells. MATERIAL AND METHODS: We used HLA-A2 **HA-1**/HA-2 tetramers (**HA-1**(A2)/HA-2(A2) tetramers) to monitor the

in vitro generation of **HA-1**- or HA-2-specific **cytotoxic T cells**. RESULTS: We show that the intensity of the tetramer-staining of the **HA-1** /HA-2-specific **cytotoxic T cells** strongly correlates with their capability to recognize mHag positive target cells. The bright tetramer-staining **cytotoxic T cells** lyse target cells expressing the natural ligand. The dim tetramer-staining **cytotoxic T cells** fail to lyse natural ligand positive target cells and lyse **peptide** -pulsed target cells only. The frequency of bright tetramer-staining, high avidity minor histocompatibility antigen-specific CTLs increases significantly upon appropriate antigen-specific restimulations. CONCLUSION: Our results demonstrate that HLA class I-minor histocompatibility antigen tetramers are useful tools for monitoring and selection of high avidity **HA-1**- and HA-2-specific **cytotoxic T cells** for adoptive immunotherapy.

L12 ANSWER 19 OF 26 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:293531 Document No.: PREV200100293531. Effect of disparity in the newly identified minor histocompatibility antigen SKH13 on the development of graft-versus-host disease after marrow transplantation from an HLA-identical sibling. Akatsuka, Yoshiki [Reprint author]; Warren, Edus H.; Brickner, Anthony G.; Lin, Ming-Tseh; Gooly, Ted; Martin, Paul J.; Hansen, John A.; Engelhard, Victor H.; Riddell, Stanley R.. Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 202a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB We have identified a new HLA*0201-restricted minor histocompatibility antigen encoded by the KIAA0020 gene and recognized by CD8+ **cytotoxic T cells** (CTL) derived from a patient with chronic GVHD (Brickner et al, submitted). This antigen, termed HA-8, results from a proline (P) to arginine (R) substitution at position 149 of the KIAA0020 protein (position 1 of the antigenic epitope). **Peptides** containing both R and P at position 1 bind HLA A201 when pulsed onto cells in vitro but expression of minigene constructs encoding these **peptides** demonstrated that only the **peptide** containing R is appropriately processed and transported into the endoplasmic reticulum. KIAA0020 is broadly expressed in tissues with the highest levels in lung and liver. A PCR-RFLP method for genotyping KIAA0020 was developed and a retrospective analysis was performed to evaluate the effect of HA-8 disparity on GVHD after HLA identical sibling transplant. Genomic DNA samples from 235 Caucasian donor/recipient pairs previously used for the analysis of **HA-1** disparity (Tseng et al, Blood 94: 2911, 1999) were used for this study. All patients received methotrexate and cyclosporin for GVHD prophylaxis. Of 235 patients, 25 (10.6%) received an HA-8 incompatible transplant and 210 (89.4%) received an HA-8 compatible transplant. Grade II - IV acute GVHD occurred in 12 (48.0%) of the HA-8 incompatible and 45.2 % of the HA-8 compatible recipients (p=.79). Clinical or pathologic chronic GVHD was diagnosed in 18/25 (72%) of incompatible recipients compared with 114/210 (54%) compatible recipients (p=.09). These results suggest a potential association of HA-8 disparity with cGVHD. An HLA A2/HA-8 tetramer has been constructed and is being used prospectively to identify HA-8 specific T cells in blood and tissues after allogeneic BMT.

L12 ANSWER 20 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN

1999:96270 Document No. 130:167163 The **HA-1** antigen.
Goulmy, Elsa Afra Julia Maria; Hunt, Donald Frederick; Engelhard, Victor Henry (Rijksuniversiteit te Leiden, Neth.). PCT Int. Appl. WO 9905173 A1 19990204, 57 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU,

ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-NL424 19980723.

AB The present invention discloses the **peptide** sequence of a so-called minor H antigen. The minor H antigens are associated with the graft vs. host disease. The **peptide** and its derivs. find many uses in bone marrow transplantation, organ transplantation and in the treatment of leukemia. The **peptide** and its derivs. can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits. The **peptide** is derived from the **HA-1** minor antigen and has the sequence VLXDDLLEA, wherein X represents a histidine or an arginine residue. Both donors and recipients in bone marrow transplantation can be treated with the **peptides**, optionally in combination with other **peptides**, coupled to carriers, with suitable excipients and/or adjuvants.

L12 ANSWER 21 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN

1999:797062 Document No. 132:92049 Induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** for the treatment of leukemia after allogeneic stem cell transplantation. Reply to comments. Mutis, T.; Goulmy, E. (Department of Immunohematology and Blood Bank, Leiden University Medical Center, Leiden, Neth.). Blood, 94(12), 4376 (English) 1999. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: W. B. Saunders Co..

AB A polemic in response to P. Brossart et al. (ibid, 4374) on evidence that HA-1H **peptide**-specific **cytotoxic T-cells** (CTL) induced in vitro using HA-1H **peptide**-pulsed monocyte-derived dendritic cells as APC from unprimed **HA-1**-neg. healthy donors are not only able to lyse primary leukemic blasts or immortalized B cells naturally expressing the HA-1H/H phenotype but also recognize heterozygous leukemic cells with the HA-1H/R phenotype, showing that these **HA-1**-specific CTL are of high affinity to the **peptide**/MHC complex.

L12 ANSWER 22 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN

1999:797061 Document No. 132:92048 Induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** for the treatment of leukemia after allogeneic stem cell transplantation. Comments. Brossart, Peter; Spahlinger, Brigitte; Grunebach, Frank; Stuhler, Gernot; Reichardt, Volker L.; Kanz, Lothar; Brugger, Wolfram (Department of Hematology, Oncology and Immunology, University of Tubingen, Tubingen, Germany). Blood, 94(12), 4374-4376 (English) 1999. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: W. B. Saunders Co..

AB A polemic in response to T. Mutis et al. (Blood 1999, 93, 2336) on evidence that HA-1H **peptide**-specific **cytotoxic T-cells** (CTL) induced in vitro using HA-1H **peptide**-pulsed monocyte-derived dendritic cells as APC from unprimed **HA-1**-neg. healthy donors are not only able to lyse primary leukemic blasts or immortalized B cells naturally expressing the HA-1H/H phenotype but also recognize heterozygous leukemic cells with the HA-1H/R phenotype, showing that these **HA-1**-specific CTL are of high affinity to the **peptide**/MHC complex.

L12 ANSWER 23 OF 26 MEDLINE on STN DUPLICATE 9

1999013046. PubMed ID: 9798702. **HA-1** and the SMCY-derived **peptide** FIDSYICQV (H-Y) are immunodominant minor histocompatibility antigens after bone marrow transplantation. Rufer N; Wolpert E; Helg C; Tiercy J M; Gratwohl A; Chapuis B; Jeannet M; Goulmy E; Roosnek E. (Department of Internal Medicine, University Hospital, Geneva, Switzerland.) Transplantation, (1998 Oct 15) 66 (7) 910-6. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Allogeneic bone marrow donors can be incompatible at different levels. Even HLA-identical pairs will be still incompatible for numerous minor histocompatibility antigens (mHag). Nevertheless, some incompatibilities are found to be associated with an increased risk of graft-versus-host disease (GVHD), which could be related to the way the immune system recognizes these antigens. METHODS: We determined the specificity of **cytotoxic T-cell** clones isolated during acute GVHD or during bone marrow graft rejection in patients (n=14) transplanted with marrow from donors who were histoincompatible for different minor and/or major histocompatibility antigens. RESULTS: We found a clear hierarchy among the different types of histoincompatibilities. In three combinations mismatched for a class I allele, all 27 clones isolated during GVHD were specific for the incompatible HLA molecule. In the 11 class I-identical combinations, 14 different mHags were recognized. The mHag **HA-1**, known to have a significant impact on the development of GVHD, was recognized in the two **HA-1**-incompatible combinations. In one of these combinations, which was sex mismatched, all 56 clones analyzed were directed against **HA-1**, demonstrating the dominance of this mHag. In the four **HA-1**-compatible, sex-mismatched combinations, the anti-H-Y response was directed against one immunodominant epitope rather than against multiple Y-chromosome-encoded epitopes. All male specific cytotoxic T lymphocytes (n=15) recognized the same high-performance liquid chromatography-purified **peptide** fraction presented by T2 cells. Moreover, all cytotoxic T lymphocytes tested (n=6) were specific for the SMCY-derived **peptide** FIDSYICQV, originally described as being the H-Y epitope recognized in the context of HLA-A*0201. CONCLUSIONS: Some histocompatibility antigens are recognized in an immunodominant fashion and will therefore be recognized in the majority of mismatched combinations. Only for such antigens, correlations between mismatches and the occurrence of GVHD or graft rejections will be found.

L12 ANSWER 24 OF 26 MEDLINE on STN DUPLICATE 10

1999036482. PubMed ID: 9820596. Genomic identification of the minor histocompatibility antigen **HA-1** locus by allele-specific PCR. Wilke M; Pool J; den Haan J M; Goulmy E. (Department of Immunohematology and Bloodbank, Leiden University Medical Center, The Netherlands.. ihbsecr@euronet.nl) . Tissue antigens, (1998 Oct) 52 (4) 312-7. Journal code: 0331072. ISSN: 0001-2815. Pub. country: Denmark. Language: English.

AB Graft-versus-host disease (GvHD) can be a major complication of allogeneic bone marrow transplantation even in recipients of HLA genotype-identical transplants. Disparities in minor histocompatibility antigens (mHags) between donor and recipient are a potential risk for the development of GvHD. A mismatch for the mHag **HA-1** can cause GvHD in adult recipients of allogeneic bone marrow from HLA-identical donors. The mHag **HA-1**, first identified by HLA-A*0201-restricted **cytotoxic T cells** (CTLs), was recently chemically characterized as a nonapeptide. On the cDNA level, the **HA-1** locus has two alleles, HA-1H and HA-1R, which differ in two nucleotides, resulting in a single amino acid substitution. Here we report on the genomic structure of the **HA-1** locus. Isolation and sequencing of cosmid DNA encoding the **HA-1 peptide** sequence revealed that the **HA-1** alleles are encoded by two exons. Two different primer sets were designed, each consisting of allele-specific primers and a common primer, and both sets containing intronic sequences. We performed genomic DNA typing of three families consisting of 24 HLA-A*0201-positive individuals. The predicted allele-specific products correlated in all cases with the mHag classification by CTLs and by RT-PCR. We demonstrate for the first time the genomic identification of the mHag **HA-1** locus. Prospective genomic typing for the **HA-1** alleles will improve donor selection and identify HLA-A*0201-positive recipients with a high risk for **HA-1**

-induced GvHD.

L12 ANSWER 25 OF 26 MEDLINE on STN DUPLICATE 11
97080610. PubMed ID: 8921955. Conservation of minor histocompatibility antigens between human and non-human primates. den Haan J M; Bontrop R E; Pool J; Sherman N; Blokland E; Engelhard V H; Hunt D F; Goulmy E. (Department of Immunohaematology and Bloodbank, Leiden University Hospital, The Netherlands.. haan.j@rulgca.leidenuniv.nl) . European journal of immunology, (1996 Nov) 26 (11) 2680-5. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB It is well accepted that minor histocompatibility antigens (mHag) can function as transplantation barriers between HLA-matched individuals. Little is known about the molecular nature and evolutionary conservation of mHag. It is only very recently that the first human mHag were identified. The HLA-A2.1-restricted mHag HA-2 and the HLA-B7-restricted mHag H-Y appeared to be **peptides** derived from polymorphic self proteins. Here we show that the HLA-A2.1-restricted mHag **HA-1**, HA-2, and the H-Y **peptides** are conserved between man, chimpanzees and rhesus macaques. Human **cytotoxic T cell** clones specific for the HLA-A2.1-restricted mHag **HA-1**, HA-2, and H-Y recognized HLA-A2.1 gene-transfected chimpanzee and rhesus macaque cells. High-pressure liquid chromatography fractionation of HLA-A2.1-bound **peptides** isolated from the HLA-A2.1-transfected chimpanzee cells revealed that the chimpanzee **HA-1** and HA-2 co-eluted with the human **HA-1** and HA-2. Subsequent amino acid sequencing showed that the chimpanzee HA-2 **peptide** is identical to the human HA-2 **peptide.** Our functional and biochemical results demonstrate that mHag **peptides** are conserved for over 35 million years.

L12 ANSWER 26 OF 26 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
95:312880 The Genuine Article (R) Number: QW602. INTERINDIVIDUAL CONSERVATION OF T-CELL RECEPTOR-BETA CHAIN VARIABLE REGIONS BY MINOR HISTOCOMPATIBILITY ANTIGEN-SPECIFIC HLA-A-ASTERISK-0201-RESTRICTED **CYTOTOXIC T-CELL** CLONES. GOULMY E (Reprint); POOL J; VANDENELSEN P J. UNIV LEIDEN HOSP, DEPT IMMUNOHAEMATOL, BLDG 1-E3Q, POB 9600, 2300 RC LEIDEN, NETHERLANDS (Reprint); UNIV LEIDEN HOSP, BLOOD BANK, 2300 RC LEIDEN, NETHERLANDS. BLOOD (01 MAY 1995) Vol. 85, No. 9, pp. 2478-2481. ISSN: 0006-4971. Pub. country: NETHERLANDS. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Minor histocompatibility antigens (mHags) are involved in the induction of graft-versus-host disease (GVHD) after HLA-identical bone marrow transplantation. Previously, we isolated a series of HLA-A*0201-restricted **cytotoxic T-cell** (CTL) clones specific for the same mHag **HA-1** from peripheral blood of three unrelated patients who were suffering from GVHD, We have now analyzed the composition of the T-cell receptor (ICR) V regions of 12 of these mHag **HA-1**-specific HLA-A*0201-restricted CTL clones by DNA sequencing of the alpha and beta chains, Of these 12 clones, derived from three unrelated individuals, five independent TCR alpha V- and beta V-region sequences were established, The TCR alpha chains were composed of varying TCR alpha V and TCR alpha J genes with no obvious similarities in structure in the N regions. However, the TCR beta chains all used the TCR beta V6S9 gene segment, and showed remarkable similarities within the N-D-N regions; ie, three independent beta-chain sequences (originating from donors Ha and Gy) shared a leucine/valine amino acid pair, whereas the other two (originating from donors Ha and Wi) shared a serine/threonine pair, all at positions 99 and 100 of the TCR beta V region, In conclusion, the TCR analysis of **HA-1** mHag-specific CTL clones has shown that the **HA-1** mHag/HLA-A*0201 complex selects for highly similar TCR beta V regions, (C) 1995 by The American Society of Hematology.

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=> d his

     (FILE 'HOME' ENTERED AT 17:22:31 ON 29 DEC 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 17:22:45 ON
     29 DEC 2004
L1             2 S "HA-1" CYTOTOXIC T CELL
L2             2 DUP REMOVE L1 (0 DUPLICATES REMOVED)
L3             0 S PROCESS GENERATING CYTOTOXIC T CELL
L4         34815 S CYTOTOXIC T CELL
L5             0 S L4 AND MINOR HISTOCOMPATIBILITY HA ANTIGEN
L6           125 S L4 AND "HA-1"
L7             0 S L6 AND "VLHDDLLEA"
L8             0 S L6 AND "VLRDDLLEA"
L9             0 S L6 AND HA PEPTIDE
L10            0 S L6 AND PULSED APC
L11           67 S L6 AND PEPTIDE
L12           26 DUP REMOVE L11 (41 DUPLICATES REMOVED)

=> s l6 and "VLXDDLLEA"
L13            1 L6 AND "VLXDDLLEA"

=> d l13 cbib abs

L13  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2004 ACS on STN
1999:96270   Document No. 130:167163  The HA-1 antigen.
     Goulmy, Elsa Afra Julia Maria; Hunt, Donald Frederick; Engelhard, Victor
     Henry (Rijksuniversiteit te Leiden, Neth.).  PCT Int. Appl. WO 9905173 A1
     19990204, 57 pp.  DESIGNATED STATES: W:  AL, AM, AT, AU, AZ, BA, BB, BG,
     BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU,
     ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM;
     RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB,
     GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1998-NL424 19980723.
AB   The present invention discloses the peptide sequence of a so-called minor
     H antigen.  The minor H antigens are associated with the graft vs. host
     disease.  The peptide and its derivs. find many uses in bone marrow
     transplantation, organ transplantation and in the treatment of leukemia.
     The peptide and its derivs. can be incorporated in vaccines, in
     pharmaceutical formulations and they can be used in diagnostic test kits.
     The peptide is derived from the HA-1 minor antigen and
     has the sequence VLXDDLLEA, wherein X represents a histidine or
     an arginine residue.  Both donors and recipients in bone marrow
     transplantation can be treated with the peptides, optionally in
     combination with other peptides, coupled to carriers, with suitable
     excipients and/or adjuvants.


=> s (goulmy e?/au or hunt d?/au or engelhard v?/au)
L14         7239 (GOULMY E?/AU OR HUNT D?/AU OR ENGELHARD V?/AU)

=> s l14 and HA-1 minor antigen CTL
L15            0 L14 AND HA-1 MINOR ANTIGEN CTL

=> s l14 and cytotoxic T cell
L16          320 L14 AND CYTOTOXIC T CELL

=> s l16 and minor H antigen
L17           12 L16 AND MINOR H ANTIGEN

=> dup remove l17
PROCESSING COMPLETED FOR L17
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L18 5 DUP REMOVE L17 (7 DUPLICATES REMOVED)

=> d l18 1-5 cbib abs

L18 ANSWER 1 OF 5 MEDLINE on STN DUPLICATE 1
2004085970. PubMed ID: 14592836. Pregnancy induces minor histocompatibility antigen-specific **cytotoxic T cells**: implications for stem cell transplantation and immunotherapy. Verdijk Rob M; Kloosterman Antoinette; Pool Jos; van de Keur Maarten; Naipal Albert M I H; van Halteren Astrid G S; Brand Anneke; Mutis Tuna; **Goulmy Els.** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.) Blood, (2004 Mar 1) 103 (5) 1961-4. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Recipients of HLA-identical stem cell transplants have a poorer transplant outcome if the donor is female rather than male. We analyzed whether pregnancy primes for minor histocompatibility (H) antigens. Peripheral blood mononuclear cells (PBMCs) from healthy multiparous female blood donors were depleted for CD4+, CD14+, CD16+, and CD19+ cells, stained with **minor H antigen**-specific HLA-A2 tetramers, sorted by fluorescence-activated cell sorting, and tested for cytotoxic activity. **Minor H antigens** HY-, HA-1-, and HA-2-specific **cytotoxic T cells** (CD8+, CD45RA-) were present in PBMCs from 4 of 7 female donors up to 22 years after the last delivery. Interestingly, in 2 of the 4 cases microchimerism of the putative immunizing **minor H antigen** was observed. Thus, pregnancy can lead to alloimmune responses against the infant's paternal **minor H antigens.** The **minor H antigen** immunization status of female donors raises important questions for the clinical practice of stem cell transplantation.

L18 ANSWER 2 OF 5 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2003:337124 Document No.: PREV200300337124. Pregnancy Induces Minor Histocompatibility Antigen Specific **Cytotoxic T Cells**: Implications for Stem Cell Transplantation and Immunotherapy. Verdijk, Rob M. [Reprint Author]; Kloosterman, Antoinette [Reprint Author]; Pool, Jos [Reprint Author]; de Keur, Maarten van [Reprint Author]; Naipal, Albert M. I. H. [Reprint Author]; Brand, Anneke [Reprint Author]; Mutis, Tuna [Reprint Author]; **Goulmy, Els A.** [Reprint Author]. Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 2426. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Recipients of HLA identical Stem Cell Transplantation (SCT) donors have a poorer transplantation outcome if the donor is female rather than male. These results can be ascribed to the effects of pregnancies leading to alloimmunization. We therefore analyzed whether pregnancy is capable of priming for minor histocompatibility (H) antigen responses. Here we demonstrate the presence of relatively high frequencies of memory, CD8+, CD45RA-, CD27-, CD62L- **cytotoxic T cells** (CTLs) specific for the **minor H antigens** HY, HA-1 and HA-2 in the PBMC of four out of eight healthy non transfused multiparous females up to 22 years after the last delivery. PBMC depleted for CD4+, CD14+ and CD19+ cells were stained with the different HLA-class I **minor H antigen** specific tetramers. A two-step FACS sort approach enabled the identification of a distinct population of tetramer+ staining cells. Frequencies of minor H specific CTL were detected between 1:3,000 to 1:23,000 within the CD8+ cell fraction. Upon non-specific in vitro stimulation these cells specifically lysed **minor H antigen** positive target cells in a cytotoxicity assay. Interestingly, in two cases hematopoietic

chimerism with cells from the putative immunizing **minor H antigen** source was observed. Thus, pregnancy can lead to cellular alloimmune response against **minor H antigens** despite concomitant HLA haplotype disparities. Immunization against **minor H antigens** through pregnancy may explain the differences in transplant outcome in both the Graft versus Host and Host versus Graft reactivities. Moreover, the fact that **minor H antigen** specific CTLs are present in healthy individuals provides a basis for **minor H antigen** vaccination strategies of healthy SC donors to enhance the efficacy of the Graft versus Tumor effect after **minor H antigen** disparate SCT.

L18 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
1999:96270 Document No. 130:167163 The HA-1 antigen. **Goulmy, Elsa Afra Julia Maria; Hunt, Donald Frederick; Engelhard, Victor Henry** (Rijksuniversiteit te Leiden, Neth.). PCT Int. Appl. WO 9905173 A1 19990204, 57 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-NL424 19980723.

AB The present invention discloses the peptide sequence of a so-called **minor H antigen.** The **minor H antigens** are associated with the graft vs. host disease. The peptide and its derivs. find many uses in bone marrow transplantation, organ transplantation and in the treatment of leukemia. The peptide and its derivs. can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits. The peptide is derived from the HA-1 minor antigen and has the sequence VLXDDLLEA, wherein X represents a histidine or an arginine residue. Both donors and recipients in bone marrow transplantation can be treated with the peptides, optionally in combination with other peptides, coupled to carriers, with suitable excipients and/or adjuvants.

L18 ANSWER 4 OF 5 MEDLINE on STN DUPLICATE 2
93278341. PubMed ID: 8504270. High frequencies of **cytotoxic T cell** precursors against minor histocompatibility antigens after HLA-identical BMT: absence of correlation with GVHD. de Bueger M; Bakker A; Bontkes H; van Rood J J; **Goulmy E.** (Department of Immunohaematology, University Hospital Leiden, The Netherlands.) Bone marrow transplantation, (1993 May) 11 (5) 363-8. Journal code: 8702459. ISSN: 0268-3369. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Limiting dilution analysis was used to quantify the frequency of **cytotoxic T cell** precursors (CTLp) against minor histocompatibility (H) antigens induced by HLA-identical BMT. The development of CTLp was monitored serially in ten patients developing either acute (n = 3), acute and chronic (n = 4) or no (n = 3) GVHD. In blood samples of patients taken shortly after BMT (< 100 days) a high frequency of anti-recipient CTLp was found (mean 1/3433). With time, this value decreased to become undetectable (< 1/500,000) beyond 400 days. This occurred also in patients still suffering from chronic GVHD. In contrast, autologous BMT did not induce any measurable recipient-reactive CTLp at any time point after BMT. In the early phase of reconstitution after BMT the frequency of CTLp against allo HLA-antigens was measured in the same patients. The absence of a consistent increase of allo-specific CTLp indicates that the kinetics of CTLp against host **minor H antigens** does not merely reflect an overall changed cytolytic potential shortly after BMT. These results indicate that: (1) HLA-identical BMT induces high frequencies of **minor H antigen**-specific CTLps detectable in the blood during the early

phase of reconstitution, and (2) the frequency of recipient-reactive CTL measured in the peripheral blood is not an adequate parameter for GVHD. These data therefore challenge the clinical value of in vitro measurement of recipient-reactive CTLs in the peripheral blood after HLA-identical sibling BMT.

L18 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
1992:39283 Document No. 116:39283 Minor histocompatibility antigens, defined by graft-vs.-host disease-derived cytotoxic T lymphocytes, show variable expression on human skin cells. De Bueger, Marleen; Bakker, Astrid; Van Rood, Jon J.; **Goulmy, Els** (Dep. Immunohaematol., Univ. Hosp. Leiden, Leiden, 2333 AA, Neth.). European Journal of Immunology, 21(11), 2839-44 (English) 1991. CODEN: EJIMAF. ISSN: 0014-2980.
AB Little is known on the effector mechanisms inducing the cutaneous lesions observed during acute graft-vs.-host disease (aGvHD) after allogeneic bone marrow transplantation (BMT). Histol. findings have indicated that infiltrating CD8+ lymphocytes probably play a role. The question was addressed of whether host minor histocompatibility (mH) antigen-reactive cytotoxic T lymphocytes (CTL) could account for this phenomenon via direct lysis of the epidermal cell layer. Six CTL clones, obtained from peripheral blood lymphocytes of patients suffering from aGvHD, each recognizing a well-characterized MHC class I-restricted mH antigen epitope, were tested on cultured keratinocytes of 9 MHC and mH antigen-typed donors. Four of 6 mH antigen-specific CTL clones lysed unstimulated MHC class I-expressing, as well as recombinant interferon-γ (rIFN-γ)-activated, ICAM-1, MHC class I- and II-expressing keratinocytes. Two strongly cytolytic CTL clones showed no recognition of keratinocytes of donors whose phytohemagglutinin-activated T cell lines were readily lysed. With respect to aGvHD, the results imply that some class I-restricted CTL obtained from peripheral blood lymphocytes of aGvHD patients have the in vitro potential to destroy resting as well as IFN-γ-activated epidermal cells, whereas others do not. In other words, CTL-defined human mH antigens vary with respect to their expression in the skin. It is intriguing that those **minor H antigens** which cannot be detected on human keratinocytes in vitro are those known to be associated with the occurrence of GvHD.

=> s l16 and HA-1
L19 100 L16 AND HA-1

=> s l19 and suicide gene
L20 0 L19 AND SUICIDE GENE

=> s l19 and expansion
L21 1 L19 AND EXPANSION

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L21 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
2004:571330 Document No. 141:258993 Artificial antigen-presenting constructs efficiently stimulate minor histocompatibility antigen-specific cytotoxic T lymphocytes. Oosten, Liesbeth E. M.; Blokland, Els; Van Halteren, Astrid G. S.; Curtsinger, Julie; Mescher, Matthew F.; Falkenburg, J. H. Frederik; Mutis, Tuna; **Goulmy, Els** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Neth.). Blood, 104(1), 224-226 (English) 2004. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.
AB Cytotoxic T lymphocytes (CTLs) specific for hematopoietic-restricted minor histocompatibility antigens (mHags) are important reagents for adoptive immunotherapy of relapsed leukemia after allogeneic stem cell transplantation. However, **expansion** of these CTLs to therapeutic nos. is often hampered by the limited supply of antigen-presenting cells (APCs). Therefore, the authors evaluated whether

cell-sized latex beads coated with HLA/mHag complexes HLA-A2/**HA-1** or HLA-A2/HA-2 and recombinant CD80 and CD54 mols. can replace professional APCs. The artificial antigen-presenting constructs (aAPCs) effectively stimulated **HA-1**-and HA-2-specific CTL clones as shown by ligand-specific **expansion**, cytokine production, and maintenance of cytotoxic activity, without alteration of CTL phenotype. Furthermore, **HA-1**-specific polyclonal CTL lines were enriched as efficiently by aAPCs as by autologous **HA-1** peptide-pulsed dendritic cells. Thus, aAPCs coated with HLA/mHag complexes, CD80, and CD54 may serve as tools for in vitro enrichment of immunotherapeutic mHag-specific CTL lines.

=> s l19 and immortalization
L22 0 L19 AND IMMORTALIZATION

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PROCESSING COMPLETED FOR L19
L23 37 DUP REMOVE L19 (63 DUPLICATES REMOVED)

=> d l23 1-37 cbib abs

L23 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
2004:392303 Document No. 140:405470 T-cell epitopes of **HA-1** minor histocompatibility antigen for immunotherapy. **Goulmy, Elsa A. J. M.; Hunt, Donald F.; Engelhard, Victor H.** (Neth.). U.S. Pat. Appl. Publ. US 2004092446 A1 20040513, 69 pp., Cont.-in-part of U.S. Ser. No. 489,760. (English). CODEN: USXXCO. APPLICATION: US 2003-623176 20030718. PRIORITY: EP 1997-202303 19970723; WO 1998-NL424 19980723; US 2000-489760 20000121.
AB The authors disclose peptides constituting T-cell epitopes of the minor histocompatibility antigen, **HA-1.** The authors demonstrate that **HA-1** is associated with graft vs. host disease, is expressed by non-hematopoietic tumor cells, and can be recognized by **HA-1 cytotoxic T cells.** The peptides and their derivs. find many uses, for instance in bone marrow transplantation, organ transplantation and in treatment of leukemia and non-hematopoietic tumors. The peptide and/or its derivs. can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits.

L23 ANSWER 2 OF 37 MEDLINE on STN DUPLICATE 1
2004085970. PubMed ID: 14592836. Pregnancy induces minor histocompatibility antigen-specific **cytotoxic T cells**: implications for stem cell transplantation and immunotherapy. Verdijk Rob M; Kloosterman Antoinette; Pool Jos; van de Keur Maarten; Naipal Albert M I H; van Halteren Astrid G S; Brand Anneke; Mutis Tuna; **Goulmy Els.** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.) Blood, (2004 Mar 1) 103 (5) 1961-4. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.
AB Recipients of HLA-identical stem cell transplants have a poorer transplant outcome if the donor is female rather than male. We analyzed whether pregnancy primes for minor histocompatibility (H) antigens. Peripheral blood mononuclear cells (PBMCs) from healthy multiparous female blood donors were depleted for CD4+, CD14+, CD16+, and CD19+ cells, stained with minor H antigen-specific HLA-A2 tetramers, sorted by fluorescence-activated cell sorting, and tested for cytotoxic activity. Minor H antigens HY-, **HA-1**-, and HA-2-specific **cytotoxic T cells** (CD8+, CD45RA-) were present in PBMCs from 4 of 7 female donors up to 22 years after the last delivery. Interestingly, in 2 of the 4 cases microchimerism of the putative immunizing minor H antigen was observed. Thus, pregnancy can lead to alloimmune responses against the infant's paternal minor H antigens. The minor H antigen immunization status of female donors raises important

questions for the clinical practice of stem cell transplantation.

L23 ANSWER 3 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
2004:571330 Document No. 141:258993 Artificial antigen-presenting constructs efficiently stimulate minor histocompatibility antigen-specific cytotoxic T lymphocytes. Oosten, Liesbeth E. M.; Blokland, Els; Van Halteren, Astrid G. S.; Curtsinger, Julie; Mescher, Matthew F.; Falkenburg, J. H. Frederik; Mutis, Tuna; **Goulmy, Els** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Neth.). Blood, 104(1), 224-226 (English) 2004. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.
AB Cytotoxic T lymphocytes (CTLs) specific for hematopoietic-restricted minor histocompatibility antigens (mHags) are important reagents for adoptive immunotherapy of relapsed leukemia after allogeneic stem cell transplantation. However, expansion of these CTLs to therapeutic nos. is often hampered by the limited supply of antigen-presenting cells (APCs). Therefore, the authors evaluated whether cell-sized latex beads coated with HLA/mHag complexes HLA-A2/**HA-1** or HLA-A2/HA-2 and recombinant CD80 and CD54 mols. can replace professional APCs. The artificial antigen-presenting constructs (aAPCs) effectively stimulated **HA-1**-and HA-2-specific CTL clones as shown by ligand-specific expansion, cytokine production, and maintenance of cytotoxic activity, without alteration of CTL phenotype. Furthermore, **HA-1**-specific polyclonal CTL lines were enriched as efficiently by aAPCs as by autologous **HA-1** peptide-pulsed dendritic cells. Thus, aAPCs coated with HLA/mHag complexes, CD80, and CD54 may serve as tools for in vitro enrichment of immunotherapeutic mHag-specific CTL lines.

L23 ANSWER 4 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2004:189798 The Genuine Article (R) Number: 775KG. Minor histocompatibility antigens: Allo target molecules for tumor-specific immunotherapy. **Goulmy E (Reprint)**. Leiden Univ, Med Ctr, Dept Immunohaematol & Bloodtransfus, Post Box 9600, NL-2300 RC Leiden, Netherlands (Reprint); Leiden Univ, Med Ctr, Dept Immunohaematol & Bloodtransfus, NL-2300 RC Leiden, Netherlands. CANCER JOURNAL (JAN-FEB 2004) Vol. 10, No. 1, pp. 1-7 . Publisher: JONES AND BARTLETT PUBLISHERS. 40 TALL PINE DR, SUDBURY, MA 01776 USA. ISSN: 1528-9117. Pub. country: Netherlands. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB Minor histocompatibility antigens have to be considered as key molecules in the stem cell-based immunotherapy of malignancies. Allogeneic stem cell transplantation (SCT) is a well-established and effective therapy for advanced hematologic malignancies. The apparent powerful graft-versus-leukemia effect of SCT led clinicians to apply SCT for the treatment of metastatic solid tumors. The SCT-based graft-versus-tumor reaction in the allogeneic human leukocyte antigen-matched SCT setting is mediated by allo-immune effector cells directed against tumor-related target antigens. The target molecules involved in the allo-immune graft-versus-tumor reaction are tumor-specific antigens, tumor-associated antigens, and tissue- and cell-specific minor histocompatibility antigens. The power of the minor histocompatibility antigens in the human leukocyte antigen-identical, stem cell-based immunotherapy for malignancies is their "allo-ness." As opposed to tumor-associated self antigens, the complexes of MHC and allo-target peptide are likely to be more immunogeneic than the major histocompatibility complex and self-target peptide complexes. Moreover, minor histocompatibility allo-antigens are not subject to self tolerance. Earlier minor histocompatibility antigens were seen as alien entities, disturbing the success of the so ideally matched organ and SCT donor-recipient combinations. To date, minor histocompatibility antigens can be set in the favorable light of useful tools for immunotherapy for cancer. The first clinical application of the hematopoietic minor histocompatibility antigens **HA-1** and HA2 is currently being explored in a stem cell-based setting for hematologic malignancies. Because **HA-1** is also expressed on carcinoma cells, a

stem cell-based vaccination trial for patients with metastatic breast or renal cancer is about to start as well. The immunotherapeutic potential of minor histocompatibility antigens demands serious searches for new minor histocompatibility antigens and analyses of their phenotype frequency, tissue distribution, and functional membrane expression. The minor histocompatibility antigens meeting the prerequisites for specific immunotherapy for malignancies, such as membrane expression and tissue and/or cell specificity, may offer the curative tools for stem cell-based immunotherapy for various hematologic and nonhematologic malignancies.

L23 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
2003:450776 Document No. 139:21025 Minor histocompatibility antigen **HA-1**: target antigen for immunotherapy of tumors.
Goulmy, Elsa Afra Julia Maria (Academisch Ziekenhuis Leiden, Neth.). Eur. Pat. Appl. EP 1317927 A1 20030611, 21 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-204704 20011205.

AB Allogeneic stem cell transplants (SCT) can induce curative Graft vs. Tumor (GvT) reactivities in patients with hematol. malignancies. The GvT reaction is mainly mediated by allo immune donor T-cells specific for polymorphic minor Histocompatibility antigens (mHags). Among these, the mHag **HA-1** was restricted to the hematopoietic system. Here the authors report the expression of **HA-1** by non-hematopoietic tumor cells. While absent in normal epithelial cells, tumor cells, tumor cell lines particularly from epithelial origin, also express **HA-1** and are recognized by **HA-1 cytotoxic T cells.** The invention provides, among others, means and methods for **HA-1** specific immunotherapy for **HA-1** pos. patients with non-hematopoietic tumor cells.

L23 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 2
2003440516. PubMed ID: 14502255. Quantification of the **HA-1** gene product at the RNA level; relevance for immunotherapy of hematological malignancies. Wilke Martina; Dolstra Harry; Maas Frans; Pool Jos; Brouwer Rolf; Falkenburg J H Frederik; Rebello Ashok; Lamers Femke; Schuuring Ed; Kluin Philip; Brasseur Francis; **Goulmy Els.** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.) hematology journal : official journal of the European Haematology Association / EHA, (2003) 4 (5) 315-20. Journal code: 100965523. ISSN: 1466-4860. Pub. country: England: United Kingdom. Language: English.

AB Minor histocompatibility antigens can induce **cytotoxic T cells** that play an important role in the graft-versus-leukemia and graft-versus-host-disease (GvHD) activity after stem cell transplantation. Minor histocompatibility antigens (mHags) with expression limited to the hematopoietic system may have a prominent role in the graft-versus-leukemia reaction. Earlier in vitro studies demonstrated that **cytotoxic T cells** specific for the minor histocompatibility antigen **HA-1** only lysed cells of hematopoietic origin. Despite this limited expression, an **HA-1** mismatch is associated with GvHD. Yet, the hematopoietic-restricted **HA-1** membrane expression motivated us to develop an ex vivo **HA-1**-specific protocol for cellular immunotherapy of relapsed leukemia. To ensure the feasibility and safety of such cellular therapy, broad **HA-1** RNA analysis is indispensable. Here we demonstrate the hematopoietic-restricted expression at the **HA-1** gene transcriptional level with high RNA expression in normal and in malignant hematopoietic cells and background expression levels in nonhematopoietic cells. In tissues that showed low **HA-1** RNA expression, hematopoietic cells were present as demonstrated by CD45 RNA expression analyzed in parallel. Thus, the mHag **HA-1** can function as an excellent target antigen for immunotherapy of hematological malignancies with no or

low risk of GvHD.

L23 ANSWER 7 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
2003:64824 Document No. 139:5250 Competition-based cellular peptide binding assays for 13 prevalent HLA class I alleles using fluorescein-labeled synthetic peptides. Kessler, Jan H.; Mommaas, Bregje; Mutis, Tuna; Huijbers, Ivo; Vissers, Debby; Benckhuijsen, Willemien E.; Schreuder, Geziena M. Th.; Offringa, Rienk; **Goulmy, Els**; Melief, Cornelis J. M.; van der Burg, Sjoerd H.; Drijfhout, Jan W. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, 2300 RC, Neth.). Human Immunology, 64(2), 245-255 (English) 2003. CODEN: HUIMDQ. ISSN: 0198-8859. Publisher: Elsevier Science Inc..
AB We report the development, validation, and application of competition-based peptide binding assays for 13 prevalent human leukocyte antigen (HLA) class I alleles. The assays are based on peptide binding to HLA mols. on living cells carrying the particular allele. Competition for binding between the test peptide of interest and a fluorescein-labeled HLA class I binding peptide is used as read out. The use of cell membrane-bound HLA class I mols. circumvents the need for laborious biochem. purification of these mols. in soluble form. Previously, we have applied this principle for HLA-A2 and HLA-A3. We now describe the assays for HLA-A1, HLA-A11, HLA-A24, HLA-A68, HLA-B7, HLA-B8, HLA-B14, HLA-B35, HLA-B60, HLA-B61, and HLA-B62. Together with HLA-A2 and HLA-A3, these alleles cover more than 95% of the Caucasian population. Several allele-specific parameters were determined for each assay. Using these assays, we identified novel HLA class I high-affinity binding peptides from HIVpol, p53, PRAME, and minor histocompatibility antigen **HA-1**. Thus, these convenient and accurate peptide-binding assays will be useful for the identification of putative cytotoxic T lymphocyte epitopes presented on a diverse array of HLA class I mols.

L23 ANSWER 8 OF 37 MEDLINE on STN DUPLICATE 3
2002459659. PubMed ID: 12218130. Identification of a novel HLA-B60-restricted T cell epitope of the minor histocompatibility antigen **HA-1** locus. Mommaas Bregje; Kamp Janine; Drijfhout Jan-Wouter; Beekman Nico; Ossendorp Ferry; Van Veelen Peter; Den Haan Joke; **Goulmy Els**; Mutis Tuna. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.) Journal of immunology (Baltimore, Md. : 1950), (2002 Sep 15) 169 (6) 3131-6. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB The polymorphic minor histocompatibility Ag **HA-1** locus encodes two peptides, **HA-1**(H) and **HA-1**(R), with a single amino acid difference. Whereas the immunogenicity of the **HA-1**(R) allele has not yet been shown, the nonameric **HA-1**(H) peptide induces HLA-A2-restricted **cytotoxic T cells** in vivo and in vitro. It is not known whether the mHag **HA-1** (H) or **HA-1**(R) associates with other HLA class I molecules. Therefore, the polymorphic regions of both **HA-1** alleles were analyzed to identify HLA class I binding peptides that are properly processed by proteasomal degradation. Peptide binding analyses were performed for all nonameric **HA-1**(H/R) peptides for binding to nine HLA class I molecules with >10% prevalence in the Caucasian population and for seven nonameric/decameric **HA-1**(H/R) peptides predicted to bind to HLA-A3, -B14, and -B60. Only the nonameric KECVL(H)/(R)DDL and decameric KECVL(H)/(R)DDLL peptides showed strong and stable binding to HLA-B60. In vitro digestion of 29-aa-long **HA-1** peptides by purified 20S proteasomes revealed proper cleavage at the COOH termini of both HLA-B60 binding **HA-1**(H) and **HA-1**(R) peptides. In subsequent analyses, dendritic cells pulsed with the nonameric **HA-1**(R) peptide did not induce CTLs that recognize the natural HLA-B60/**HA-1**(R) ligand. In contrast, dendritic cells

pulsed with the nonameric **HA-1**(H) peptide induced IFN-gamma-secreting T cells specific for the natural HLA-B60/**HA-1**(H) ligand in three HLA-B60(+) **HA-1**(RR) individuals, demonstrating the immunogenicity of the HLA-B60/**HA-1**(H) ligand. In conclusion, this study shows a novel HLA-B60-restricted T cell epitope of the minor histocompatibility Ag **HA-1** locus.

L23 ANSWER 9 OF 37 MEDLINE on STN DUPLICATE 4
2002080614. PubMed ID: 11807003. General T-cell receptor antagonists to immunomodulate HLA-A2-restricted minor histocompatibility antigen **HA-1**-specific T-cell responses. den Haan Joke M M; Mutis Tuna; Blokland Els; IJzerman Ad P; **Goulmy Els.** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden University, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.) Blood, (2002 Feb 1) 99 (3) 985-92. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB T-cell receptors (TCRs) of a series of minor histocompatibility antigen (mHag) **HA-1**-specific **cytotoxic T-cell** (CTL) clones isolated from 3 unrelated patients have been shown to use the same BV6S4A2 segment with conserved amino acids in the CDR3Vbeta region. This suggests that different **HA-1**-specific TCRs interact similarly to the **HA-1** antigen presented by the HLA-A2 molecule. The mHag **HA-1** forms an immunogenic complex with HLA-A2 and induces strong alloimmune responses after stem cell transplantation (SCT). It was questioned, therefore, whether clonal and polyclonal **HA-1**-specific CTL responses can be antagonized by a single TCR antagonistic peptide. Functional analysis and molecular modeling of single and double amino acid substitutions of TCR contact residues, adjacent residues, and HLA-A2 binding residues resulted in 4 peptides with high affinity for HLA-A2 and with the capacity to inhibit the lysis of endogenously **HA-1**-expressing EBV-BLCL by 3 different **HA-1**-specific CTL clones. These peptides also efficiently antagonized **HA-1**-specific polyclonal CTL lines derived from 3 patients and significantly reduced the number of interferon-gamma-producing **HA-1**-specific CTL of a patient with graft-versus-host disease after **HA-1**-mismatched SCT. These data show that general TCR antagonists can be developed that inhibit HLA-A2-restricted **HA-1**-specific CTL responses on the clonal and the polyclonal level and that TCR antagonists may modulate the immunodominant mHag **HA-1** responses.

L23 ANSWER 10 OF 37 MEDLINE on STN DUPLICATE 5
2002348185. PubMed ID: 12091347. Generation of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** restricted by nonself HLA molecules: a potential strategy to treat relapsed leukemia after HLA-mismatched stem cell transplantation. Mutis Tuna; Blokland Els; Kester Michel; Schrama Ellen; **Goulmy Els** . (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands.. t.mutis@lumc.nl) . Blood, (2002 Jul 15) 100 (2) 547-52. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Successful stem cell transplantation (SCT) across HLA barriers can be performed with cord blood, megadoses of stem cells, or with nonmyeloablative conditioning strategies. Because the HLA-mismatched transplants are often T-cell depleted, leukemia relapse rates are high. Treatment of relapsed leukemia after HLA-mismatched SCT is difficult. A novel potential strategy to treat relapsed leukemia after HLA-mismatched SCT is the use of patients' mismatched HLA molecules as antigen-presenting molecules to generate hematopoietic system-specific **cytotoxic T cells** (CTLs) from the stem cell donor. Adoptive transfer of these hematopoietic system-specific CTLs that are restricted by nonself HLA molecules may eliminate leukemia without affecting the patient's nonhematopoietic cells or donor hematopoietic cells. We

investigated the feasibility of this strategy using the hematopoietic system-specific minor histocompatibility antigen **HA-1**, which is known to induce HLA-A2-restricted CTLs. HLA-A2(-) peripheral blood mononuclear cells were stimulated with HLA-A2(+) T2 cells pulsed with synthetic **HA-1** peptide or with dendritic cells transduced with the **HA-1** cDNA. Tetrameric HLA-A2/**HA-1** peptide complexes were used to monitor and enrich **HA-1**-specific CTLs. In the alloreactive cultures, **HA-1**-specific CTLs were enriched up to 7% by 3 rounds of antigen-specific stimulations and up to 87% by fluorescence-activated cell sorting of tetramer-positive T cells. The **HA-1** -specific CTLs showed specific lysis of the relevant target cells, including leukemic cells. Because the polyclonal CTL cultures also contained natural killer cells and allo-HLA-A2-specific CTLs, CTL clones were generated that showed the expected **HA-1** specificity only. Thus, **HA-1**-specific CTLs restricted by nonself HLA-A2 molecules can be generated in an HLA-A2-mismatched setting.

L23 ANSWER 11 OF 37 MEDLINE on STN DUPLICATE 6
2002472540. PubMed ID: 12234166. Efficient induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T-cells** using dendritic cells retrovirally transduced with **HA-1**-coding cDNA. Mutis Tuna; Ghoreschi Kamran; Schrama Ellen; Kamp Janine; Heemskerk Mirjam; Falkenburg J H Frederik; Wilke Martina; **Goulmy Els.** (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands.. t.mutis@lumc.nl) . Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, (2002) 8 (8) 412-9. Journal code: 9600628. ISSN: 1083-8791. Pub. country: United States. Language: English.

AB **Cytotoxic T-cells** (CTLs) specific for the hematopoietic system-restricted minor histocompatibility antigen (mHag) **HA-1** efficiently lyse **HA-1**-positive leukemic cells without affecting nonhematopoietic cells. **HA-1**-specific CTLs are thus potential tools for adoptive immunotherapy of relapsed leukemia after HLA-matched-**HA-1**-mismatched stem cell transplantation (SCT). In vitro generation of **HA-1**-specific CTLs from SC donors is possible using dendritic cells (DCs) pulsed with synthetic **HA-1** peptide as stimulator cells. However, this approach requires at least 6 weeks of in vitro culturing under GMP (good manufacturing practice) conditions. Our data show that in vitro induction of **HA-1**-specific CTLs is more rapid with the use of DCs that are retrovirally transduced with the **HA-1** complementary DNA. Retrovirally transduced DCs showed functional and long-term stable expression of the **HA-1** CTL epitope in primary CTL cultures. In 4 SC donors, **HA-1**-transduced DCs induced **HA-1**-specific CTLs in 14 to 21 days. The in vitro-generated CTL lines contained 6% to 9% T-cells that stained brightly with tetrameric HLA-A2/**HA-1** peptide complexes (**HA-1**(A2) tetramer) and showed significant lysis of **HA-1**+ leukemic cells. The CTL induction procedure using peptide-pulsed DCs was less effective and required 28 to 35 days of T-cell culture. Thus, sustained presentation of mHag **HA-1** by retrovirally transduced DCs facilitates the in vitro induction of **HA-1**-specific CTLs.

L23 ANSWER 12 OF 37 MEDLINE on STN DUPLICATE 7
2002194878. PubMed ID: 11927949. In situ dissection of the graft-versus-host activities of **cytotoxic T cells** specific for minor histocompatibility antigens. Dickinson Anne M; Wang Xiao-Nong; Sviland Lisbet; Vyth-Dreese Florry A; Jackson Graham H; Schumacher Ton N M; Haanen John B A G; Mutis Tuna; **Goulmy Els.** (University Department of Haematology, Royal Victoria Infirmary,

University of Newcastle, Newcastle upon Tyne, UK.) Nature medicine, (2002 Apr) 8 (4) 410-4. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB Minor histocompatibility antigens (mHags) are immunogenic peptides from polymorphic cellular proteins that induce strong T-cell responses after human leukocyte antigen (HLA)-matched, mHag-mismatched stem-cell transplantation. mHags with broad or limited tissue expression are target antigens for graft-versus-host (GvH) and graft-versus-leukemia (GvL) reactivities. Separation of these activities is crucial for adoptive immunotherapy of leukemia without GvH disease. Therefore, using a skin-explant assay we investigated the in situ activities of cytotoxic T lymphocytes (CTLs) specific for the ubiquitously expressed mHag H-Y and for the hematopoietic-restricted mHags **HA-1** and HA-2. H-Y-specific CTLs, visualized by tetrameric HLA-mHag peptide complexes, infiltrated male skin sections within 24 hours, induced severe GvH reactions of grade III-IV and produced high levels of IFN-gamma. In contrast, CTLs specific for the hematopoietic system-specific mHags **HA-1** and HA-2 induced no or low GvH reactions above background and produced little or no interferon-gamma, unless the skin sections were preincubated with **HA-1**/HA-2 synthetic peptides. These results provide the first in situ dissection of GvH effects by mHag-specific CTLs and show that ubiquitously expressed mHags are the prime targets of GvH disease.

L23 ANSWER 13 OF 37 MEDLINE on STN DUPLICATE 8

2002425399. PubMed ID: 12163564. The hematopoietic system-specific minor histocompatibility antigen **HA-1** shows aberrant expression in epithelial cancer cells. Klein Christoph A; Wilke Martina; Pool Jos; Vermeulen Corine; Blokland Els; Burghart Elke; Krostina Sabine; Wendler Nicole; Passlick Bernward; Riethmueller Gert; **Goulmy Els** . (Department of Immunology, Klinikum Innenstadt, Ludwig-Maximilians University, 80336 Munich, Germany.. E.A.J.M.Goulmy@lumc.nl) . Journal of experimental medicine, (2002 Aug 5) 196 (3) 359-68. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Allogeneic stem cell transplantation (SCT) can induce curative graft-versus-tumor reactions in patients with hematological malignancies and solid tumors. The graft-versus-tumor reaction after human histocompatibility leukocyte antigen (HLA)-identical SCT is mediated by alloimmune donor T cells specific for polymorphic minor histocompatibility antigens (mHags). Among these, the mHag **HA-1** was found to be restricted to the hematopoietic system. Here, we report on the **HA-1** ribonucleic acid expression by microdissected carcinoma tissues and by single disseminated tumor cells isolated from patients with various epithelial tumors. The **HA-1** peptide is molecularly defined, as it forms an immunogenic peptide ligand with HLA-A2 on the cell membrane of carcinoma cell lines. **HA-1**-specific **cytotoxic T cells** lyse epithelial tumor cell lines in vitro, whereas normal epithelial cells are not recognized. Thus, **HA-1**-specific immunotherapy combined with HLA-identical allogeneic SCT may now be feasible for patients with **HA-1**(+) carcinomas.

L23 ANSWER 14 OF 37 MEDLINE on STN DUPLICATE 9

2003015783. PubMed ID: 12522448. Exclusive TCRVbeta chain usage of ex vivo generated minor Histocompatibility antigen **HA-1** specific **cytotoxic T cells**: implications for monitoring of immunotherapy of leukemia by TCRBV spectratyping. Verdijk Rob M; Mutis Tuna; Wilke Martina; Pool Jos; Schrama Ellen; Brand Anneke; **Goulmy Els**. (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.. rmverdijk@lumc.nl) . hematology journal : official journal of the European Haematology Association / EHA, (2002) 3 (6) 271-5. Journal code: 100965523. ISSN: 1466-4860. Pub. country: England: United Kingdom. Language: English.

AB Tissue expression of minor Histocompatibility antigens **HA-**

1 and HA-2 is limited to the hematopoietic system. Therefore, ex vivo generated **HA-1**/HA-2 specific cytotoxic T lymphocytes (CTLs) can be applied for adoptive immunotherapy of relapsed leukemia after HLA-matched **HA-1**/HA-2 mismatched stem cell transplantation. Here we used T cell receptor beta variable chain (TCRBV) spectratyping and/or TCRBV sequencing to monitor the specific TCR usage in eleven **HA-1**/HA-2 CTLs that were induced ex vivo with peptide pulsed dendritic cells. The HA-2 induced CTLs used different TCRBV. In contrast, the development of **HA-1** specific CTLs coincided with prominent skewing of TCRBV7 spectratypes. Sequencing of the TCRBV7 specific PCR products used by these ex vivo generated **HA-1** CTLs revealed the exclusive usage of TCRBV7-9*03, identical to the TCRBV used by **HA-1** specific CTLs induced in vivo after stem cell transplantation. Thus, monitoring of immunotherapy with **HA-1** specific CTLs is now also feasible by TCRBV spectratyping.

L23 ANSWER 15 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2002:432499 The Genuine Article (R) Number: 541DL. Minor histocompatibility antigens (mHag) **HA-1** and HA-2 specific **cytotoxic T-cells** (CTL) induce complete remissions (CR) after donor lymphocyte infusion (DLI) for relapsed CML or multiple myeloma (MM) after allogeneic stem cell transplantation (alloSCT) . Marijt W A F (Reprint); Heemskerk M H M; Hoogeboom M; de Paus R A; **Goulmy E A J M**; Kester M G D; van der Hoom M A W G; Drijfhout J W; Willemze R; Falkenburg J H F. BONE MARROW TRANSPLANTATION (MAR 2002) Vol. 29, Supp. [2], pp. S2-S3. MA 76. Publisher: NATURE PUBLISHING GROUP. MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND. ISSN: 0268-3369. Language: English.

L23 ANSWER 16 OF 37 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2003:337124 Document No.: PREV200300337124. Pregnancy Induces Minor Histocompatibility Antigen Specific **Cytotoxic T Cells**: Implications for Stem Cell Transplantation and Immunotherapy. Verdijk, Rob M. [Reprint Author]; Kloosterman, Antoinette [Reprint Author]; Pool, Jos [Reprint Author]; de Keur, Maarten van [Reprint Author]; Naipal, Albert M. I. H. [Reprint Author]; Brand, Anneke [Reprint Author]; Mutis, Tuna [Reprint Author]; **Goulmy, Els A.** [Reprint Author]. Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 2426. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Recipients of HLA identical Stem Cell Transplantation (SCT) donors have a poorer transplantation outcome if the donor is female rather than male. These results can be ascribed to the effects of pregnancies leading to alloimmunization. We therefore analyzed whether pregnancy is capable of priming for minor histocompatibility (H) antigen responses. Here we demonstrate the presence of relatively high frequencies of memory, CD8+, CD45RA-, CD27-, CD62L- **cytotoxic T cells** (CTLs) specific for the minor H antigens HY, **HA-1** and HA-2 in the PBMC of four out of eight healthy non transfused multiparous females up to 22 years after the last delivery. PBMC depleted for CD4+, CD14+ and CD19+ cells were stained with the different HLA-class I minor H antigen specific tetramers. A two-step FACS sort approach enabled the identification of a distinct population of tetramer+ staining cells. Frequencies of minor H specific CTL were detected between 1:3,000 to 1:23,000 within the CD8+ cell fraction. Upon non-specific in vitro stimulation these cells specifically lysed minor H antigen positive target cells in a cytotoxicity assay. Interestingly, in two cases hematopoietic chimerism with cells from the putative immunizing minor H antigen source

was observed. Thus, pregnancy can lead to cellular alloimmune response against minor H antigens despite concomitant HLA haplotype disparities. Immunization against minor H antigens through pregnancy may explain the differences in transplant outcome in both the Graft versus Host and Host versus Graft reactivities. Moreover, the fact that minor H antigen specific CTLs are present in healthy individuals provides a basis for minor H antigen vaccination strategies of healthy SC donors to enhance the efficacy of the Graft versus Tumor effect after minor H antigen disparate SCT.

L23 ANSWER 17 OF 37 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2003:356672 Document No.: PREV200300356672. Direct Cloning of Tumor-Reactive T Cells from Peripheral Blood of Patients Treated with Donor Lymphocyte Infusion (DLI) Shows the Presence of High Numbers of Hematopoiesis-Restricted Minor Histocompatibility Antigen (mHag) **HA-1** and HA-2 Specific CD8+ T Cells. Kloosterboer, F. M. [Reprint Author]; Luxemburg-Heijs, S. A. P. van [Reprint Author]; Barbui, A. M. [Reprint Author]; Strijbosch, M. P. W. [Reprint Author]; Marijt, W. A. F. [Reprint Author]; **Goulmy, E.** [Reprint Author]; Willemze, R. [Reprint Author]; Falkenburg, J. H. F. [Reprint Author]. Department of Hematology, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 664. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Donor derived T cells recognizing mHags on recipient cells mediate the anti-leukemic effect after DLI. The hematopoiesis-restricted mHags **HA-1** and HA-2 are highly immunogenic antigens that may serve as target antigens for donor T cells. We evaluated the immunodominant nature of **HA-1** and HA-2 after DLI. First the presence of **HA-1** and HA-2 specific CD8+ T cells during the clinical response to DLI was determined. Next, the relative contribution of **HA-1** and HA-2 specific T cell clones within the total population of activated tumor-directed T cells was quantified. Three **HA-1** and HA-2 positive patients treated for relapsed multiple myeloma (patient 1) or CML (patients 2 and 3) with DLI from their HA-2 and/or **HA-1** negative donors were included in this study. **HA-1** disparity was present in all 3 HLA-genotypically identical sibling combinations. In the 3rd combination also HA-2 disparity was present. The presence of **HA-1** and HA-2 specific CD8+ T cells was measured in PB prior to and 5-7 weeks after DLI with HLA-A2/**HA-1** or HA-2 tetramers using flow cytometry. Prior to DLI the level of HA-2 and/or **HA-1** specific CTL in blood was low (ltoreq0.02%). Five to seven weeks after DLI patient 1 and 2 showed a peak in the number of **HA-1** tetramer+ T cells (0.16% and 1.57%, respectively) and patient 3 showed an increase in the number of HA-2 tetramer+ T cells (0.43%) but not in the number of **HA-1** tetramer+ T cells (0.03%). To investigate the contribution of **HA-1** and HA-2 specific T cells to the total number of tumor-reactive T cells, we isolated tumor-directed T cells from these PB samples. The PB T cells were stimulated with malignant cells isolated from the patient before transplantation. After 16 hrs of stimulation, individual cells secreting IFNgamma were isolated. Cells were labeled with an IFNgamma catch reagent, and incubated for 45 minutes at 37degreeC. After secondary labeling with PE-labeled IFNgamma detection antibody, IFNgamma secreting T cells were isolated by single cell sorting. Clonal T cells were restimulated with irradiated allogeneic feeder cells, PHA and IL2. After 2 weeks the cells were restimulated and from day 21 analyzed for cytotoxicity in a 51Cr-release assay. We generated 65, 3 and 36 **cytotoxic T cell** (CTL) clones from patient 1, 2 and 3, respectively. CTL clones lysed EBV-LCL or PHA blasts from the patient but not from the donor. HA-2 and/or **HA-1**

specificity of the CTL clones was tested by tetramer staining. 18% of the clones isolated from patient 1 were **HA-1** specific of which 77% showed high and 23% showed intermediate affinity. One of the three clones isolated from patient 2 was **HA-1** specific (high affinity). 14% of the clones isolated from patient 3 were HA-2 positive (57% high, 43% intermediate affinity). All **HA-1** or HA-2 specific CTL lysed hematopoietic cells from the patient. Cytotoxicity against the malignant cells was tested for the clones isolated from patients 2 and 3, and only the high affinity tetramer-staining CTL showed lysis of the leukemic cells. We conclude that during the clinical response to DLI from an HA-2 and/or **HA-1** disparate donor a high percentage of the tumor reactive T cells in the recipient is directed against **HA-1** or HA-2 demonstrating the immunodominant nature of these mHags.

L23 ANSWER 18 OF 37 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2002:220339 Document No.: PREV200200220339. Minor histocompatibility antigen specific CTLs and in situ skin graft versus host reactions. Wang, Xiao-Nong [Reprint author]; Dickinson, Anne M. [Reprint author]; Sviland, Lisbet; Vyth-Dreese, F. A.; Jackson, Graham H. [Reprint author]; Schumacher, Ton N. M.; Haanen, John B. A. G.; Mutis, Tuna; **Goulmy, Els.** University Department of Haematology, Royal Victoria Infirmary, Newcastle Upon Tyne, UK. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 649a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Minor Histocompatibility antigens (mHags) are immunogenic peptides from polymorphic cellular proteins that induce strong T cell responses after HLA matched mHag mismatched stem cell transplantation (SCT). mHags with broad or limited tissue expression are target antigens for Graft versus Host (GvH) or Graft versus Leukemia (GvL) reactivities, respectively. Dissection between these activities is crucial for adoptive immunotherapy of leukemia without Graft versus Host Disease (GvHD). Therefore, we investigated the in situ behaviour of **cytotoxic T cells** (CTLs) specific for the ubiquitously expressed mHag H-Y and for the hematopoietic restricted mHags **HA-1** and HA-2 in a skin explant assay. H-Y specific CTLs, visualized by tetrameric HLA/mHag peptide complexes, infiltrated male skin sections within 24 hours, induced severe GvH reactions of grade III-IV and produced high levels of IFN-gamma. In striking contrast, CTLs specific for the hematopoietic system specific mHags **HA-1** and HA-2 induced no or low GvH reactions above background and produced no or little IFN-gamma, unless the skin sections were preincubated with **HA-1**/HA-2 synthetic peptides. These results provide the first in situ dissection of GvH and GvL and prove that ubiquitously expressed mHags are the prime targets of GvHD. The results also underline that CTLs specific for the hematopoietic system specific mHags **HA-1** and HA-2 can be safely applied for the treatment of relapsed leukemia.

L23 ANSWER 19 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:401714 The Genuine Article (R) Number: 421ZZ. Interindividual conservation of T-cell receptor beta-chain variable region (tcrbv) repertoire by in vitro generated minor histocompatibility antigen **ha-1**-specific **Cytotoxic T-cells** (CTL's). Verdijk R M (Reprint); Mutis T; Kamp J; Schrama E; Brand A; Wilke M; **Goulmy E.** BONE MARROW TRANSPLANTATION (MAR 2001) Vol. 27, Supp. [1], pp. S111-S111. Publisher: NATURE PUBLISHING GROUP. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0268-3369. Language: English.

L23 ANSWER 20 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2001:401709 The Genuine Article (R) Number: 421ZZ. Efficient in vitro induction of minor histocompatibility antigen **HA-1** specific **cytotoxic T-cells** using dendritic cells retrovirally transduced with **HA-1** coding cDNA. Mutis T (Reprint); Ghoreschi K; Schrama E; Kamp J; Heemskerk M; Falkenburg J H F; **Goulmy E.** BONE MARROW TRANSPLANTATION (MAR 2001) Vol. 27, Supp. [1], pp. S110-S110. Publisher: NATURE PUBLISHING GROUP. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0268-3369. Language: English.

L23 ANSWER 21 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2001:401479 The Genuine Article (R) Number: 421ZZ. Generation of allo-HLA restricted minor histocompatibility antigen **HA-1** specific **cytotoxic T-cells** (CTLs) as tools for treatment of relapsed leukemia following HLA-mismatched stem cell transplantation. Mutis T (Reprint); Blokland E; Schrama E; **Goulmy E.** BONE MARROW TRANSPLANTATION (MAR 2001) Vol. 27, Supp. [1], pp. S1-S1. Publisher: NATURE PUBLISHING GROUP. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0268-3369. Language: English.

L23 ANSWER 22 OF 37 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2001:312013 Document No.: PREV200100312013. Efficient in vitro induction of minor histocompatibility antigen **HA-1** specific cytotoxic T-lymphocytes using dendritic cells retrovirally transduced with **HA-1** coding gene segment. Mutis, Tuna [Reprint author]; Wilke, Martina [Reprint author]; Ghoreschi, Kamran; Schrama, Ellen [Reprint author]; Kamp, Janine [Reprint author]; Heemskerk, Mirjam; Falkenburg, J. H. Frederik; **Goulmy, Els** [Reprint author]. Dept. of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 582a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.
AB The minor histocompatibility antigen (mHag) **HA-1** is a hematopoietic system specific polymorphic antigen that can be recognized by **cytotoxic T cells** (CTLs) in the context of HLA-A2. **HA-1** specific CTLs exhibit strong anti-leukemia reactivity by lysing **HA-1** positive leukemic cells and their clonogenic precursors without affecting non-hematopoietic cells. Adoptive transfer of in vitro generated **HA-1** specific CTLs into **HA-1** positive patients with relapsed leukemia may therefore be curative with a low risk of graft versus host disease (GvHD). We have recently shown the feasibility of in vitro generation of **HA-1** specific CTLs from **HA-1** negative individuals using dendritic cells (DCs) pulsed with synthetic **HA-1** peptide. However, under GMP conditions, **HA-1** CTLs can not be generated from some donors using peptide pulsed DCs. We therefore investigated whether generation of **HA-1** specific CTLs is more effective using DCs that are retrovirally transduced to express the **HA-1** antigen. The 312 base pair gene segment coding for the **HA-1** CTL epitope was cloned into the retroviral vector LZRS. This vector was transduced into several cell lines including CD34+ DC progenitors with 10-40% efficiency. All retrovirally transduced cells showed stable and functional expression of the **HA-1** CTL epitope. CD34+ DC progenitors differentiated normally into immature DCs within 10-12 days and induced strong in vitro **HA-1** specific CTL responses in four out of six **HA-1** negative healthy unprimed individuals.

The CTL lines contained 6-10% **HA-1** specific CTLs as determined by HLA-A2/**HA-1** peptide tetramers. The induction of **HA-1** specific CTLs by retrovirally transduced DCs required only one or two rounds of restimulation whereas CTL induction by peptide pulsed DCs required three to five rounds of restimulations. During the CTL induction, the retrovirally transduced DCs were detected at least seven days in the cultures and retained their immature phenotype. Our results demonstrate that retrovirally transduced immature DCs effectively induce **HA-1** specific CTL responses through their continuous presentation of the **HA-1** T cell epitope to unprimed T cell precursors.

L23 ANSWER 23 OF 37 MEDLINE on STN

2002187510. PubMed ID: 11920221. HLA class I-minor histocompatibility antigen tetramers select **cytotoxic T cells** with high avidity to the natural ligand. Gillespie G; Mutis T; Schrama E; Kamp J; Esendam B; Falkenburg J F; **Goulmy E**; Moss P. (Molecular Immunology Group, John Radcliffe Hospital, Oxford, UK.) hematology journal : official journal of the European Haematology Association / EHA, (2000) 1 (6) 403-10. Journal code: 100965523. ISSN: 1466-4860. Pub. country: England: United Kingdom. Language: English.

AB INTRODUCTION: **Cytotoxic T cells** specific for the hematopoietic system-restricted minor histocompatibility antigens **HA-1** and HA-2 are potential tools for the treatment of relapsed leukemia after minor histocompatibility antigen mismatched bone marrow transplantation. **HA-1**/HA-2-specific **cytotoxic T cells** with strong cytotoxic activity against **HA-1**/HA-2 positive target cells can be generated in vitro using **HA-1** and HA-2 peptide-pulsed dendritic cells as antigen presenting cells. MATERIAL AND METHODS: We used HLA-A2 **HA-1**/HA-2 tetramers (**HA-1**(A2)/HA-2(A2) tetramers) to monitor the in vitro generation of **HA-1**- or HA-2-specific **cytotoxic T cells.** RESULTS: We show that the intensity of the tetramer-staining of the **HA-1**/HA-2-specific **cytotoxic T cells** strongly correlates with their capability to recognize mHag positive target cells. The bright tetramer-staining **cytotoxic T cells** lyse target cells expressing the natural ligand. The dim tetramer-staining **cytotoxic T cells** fail to lyse natural ligand positive target cells and lyse peptide-pulsed target cells only. The frequency of bright tetramer-staining, high avidity minor histocompatibility antigen-specific CTLs increases significantly upon appropriate antigen-specific restimulations. CONCLUSION: Our results demonstrate that HLA class I-minor histocompatibility antigen tetramers are useful tools for monitoring and selection of high avidity **HA-1**- and HA-2-specific **cytotoxic T cells** for adoptive immunotherapy.

L23 ANSWER 24 OF 37 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:293531 Document No.: PREV200100293531. Effect of disparity in the newly identified minor histocompatibility antigen SKH13 on the development of graft-versus-host disease after marrow transplantation from an HLA-identical sibling. Akatsuka, Yoshiki [Reprint author]; Warren, Edus H.; Brickner, Anthony G.; Lin, Ming-Tseh; Gooly, Ted; Martin, Paul J.; Hansen, John A.; **Engelhard, Victor H.**; Riddell, Stanley R.. Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 202a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB We have identified a new HLA*0201-restricted minor histocompatibility antigen encoded by the KIAA0020 gene and recognized by CD8+

cytotoxic T cells (CTL) derived from a patient with chronic GVHD (Brickner et al, submitted). This antigen, termed HA-8, results from a proline (P) to arginine (R) substitution at position 149 of the KIAA0020 protein (position 1 of the antigenic epitope). Peptides containing both R and P at position 1 bind HLA A201 when pulsed onto cells in vitro but expression of minigene constructs encoding these peptides demonstrated that only the peptide containing R is appropriately processed and transported into the endoplasmic reticulum. KIAA0020 is broadly expressed in tissues with the highest levels in lung and liver. A PCR-RFLP method for genotyping KIAA0020 was developed and a retrospective analysis was performed to evaluate the effect of HA-8 disparity on GVHD after HLA identical sibling transplant. Genomic DNA samples from 235 Caucasian donor/recipient pairs previously used for the analysis of **HA-1** disparity (Tseng et al, Blood 94: 2911, 1999) were used for this study. All patients received methotrexate and cyclosporin for GVHD prophylaxis. Of 235 patients, 25 (10.6%) received an HA-8 incompatible transplant and 210 (89.4%) received an HA-8 compatible transplant. Grade II - IV acute GVHD occurred in 12 (48.0%) of the HA-8 incompatible and 45.2 % of the HA-8 compatible recipients (p=.79). Clinical or pathologic chronic GVHD was diagnosed in 18/25 (72%) of incompatible recipients compared with 114/210 (54%) compatible recipients (p=.09). These results suggest a potential association of HA-8 disparity with cGVHD. An HLA A2/HA-8 tetramer has been constructed and is being used prospectively to identify HA-8 specific T cells in blood and tissues after allogeneic BMT.

L23 ANSWER 25 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
1999:96270 Document No. 130:167163 The **HA-1** antigen.
Goulmy, Elsa Afra Julia Maria; Hunt, Donald Frederick; Engelhard, Victor Henry (Rijksuniversiteit te Leiden, Neth.). PCT Int. Appl. WO 9905173 A1 19990204, 57 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-NL424 19980723.
AB The present invention discloses the peptide sequence of a so-called minor H antigen. The minor H antigens are associated with the graft vs. host disease. The peptide and its derivs. find many uses in bone marrow transplantation, organ transplantation and in the treatment of leukemia. The peptide and its derivs. can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits. The peptide is derived from the **HA-1** minor antigen and has the sequence VLXDDLLEA, wherein X represents a histidine or an arginine residue. Both donors and recipients in bone marrow transplantation can be treated with the peptides, optionally in combination with other peptides, coupled to carriers, with suitable excipients and/or adjuvants.

L23 ANSWER 26 OF 37 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
1999:961963 The Genuine Article (R) Number: 263TD. Induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** for the treatment of leukemia after allogeneic stem cell transplantation - Response. Mutis T (Reprint); **Goulmy E.** LEIDEN UNIV, MED CTR, DEPT IMMUNOHEMATOL & BLOOD BANK, LEIDEN, NETHERLANDS (Reprint). BLOOD (15 DEC 1999) Vol. 94, No. 12, pp. 4376-4376. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0006-4971. Pub. country: NETHERLANDS. Language: English.

L23 ANSWER 27 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
1999:797062 Document No. 132:92049 Induction of minor histocompatibility

antigen **HA-1**-specific **cytotoxic T cells** for the treatment of leukemia after allogeneic stem cell transplantation. Reply to comments. Mutis, T.; **Goulmy, E.** (Department of Immunohematology and Blood Bank, Leiden University Medical Center, Leiden, Neth.). Blood, 94(12), 4376 (English) 1999. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: W. B. Saunders Co..

AB A polemic in response to P. Brossart et al. (ibid, 4374) on evidence that HA-1H peptide-specific **cytotoxic T-cells** (CTL) induced in vitro using HA-1H peptide-pulsed monocyte-derived dendritic cells as APC from unprimed **HA-1**-neg. healthy donors are not only able to lyse primary leukemic blasts or immortalized B cells naturally expressing the HA-1H/H phenotype but also recognize heterozygous leukemic cells with the HA-1H/R phenotype, showing that these **HA-1**-specific CTL are of high affinity to the peptide/MHC complex.

L23 ANSWER 28 OF 37 EMBASE on STN

1999432018 EMBASE Induction of minor histocompatibility antigen **HA-1**-specific **cytotoxic T cells** for the treatment of leukemia after allogeneic stem cell transplantation (multiple letters). Brossart P.; Spahlinger B.; Grunebach F.; Stuhler G.; Reichardt V.L.; Kanz L.; Brugger W.; Mutis T.; **Goulmy E.**. P. Brossart, Department of Hematology, University of Tubingen, Tubingen, Germany. Blood 94/12 (4374-4376) 1999.
Refs: 6.
ISSN: 0006-4971. CODEN: BLOOAW. Pub. Country: United States. Language: English.

L23 ANSWER 29 OF 37 MEDLINE on STN DUPLICATE 10

1999013046. PubMed ID: 9798702. **HA-1** and the SMCY-derived peptide FIDSYICQV (H-Y) are immunodominant minor histocompatibility antigens after bone marrow transplantation. Rufer N; Wolpert E; Helg C; Tiercy J M; Gratwohl A; Chapuis B; Jeannet M; **Goulmy E**; Roosnek E. (Department of Internal Medicine, University Hospital, Geneva, Switzerland.) Transplantation, (1998 Oct 15) 66 (7) 910-6. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Allogeneic bone marrow donors can be incompatible at different levels. Even HLA-identical pairs will be still incompatible for numerous minor histocompatibility antigens (mHag). Nevertheless, some incompatibilities are found to be associated with an increased risk of graft-versus-host disease (GVHD), which could be related to the way the immune system recognizes these antigens. METHODS: We determined the specificity of **cytotoxic T-cell** clones isolated during acute GVHD or during bone marrow graft rejection in patients (n=14) transplanted with marrow from donors who were histoincompatible for different minor and/or major histocompatibility antigens. RESULTS: We found a clear hierarchy among the different types of histoincompatibilities. In three combinations mismatched for a class I allele, all 27 clones isolated during GVHD were specific for the incompatible HLA molecule. In the 11 class I-identical combinations, 14 different mHags were recognized. The mHag **HA-1**, known to have a significant impact on the development of GVHD, was recognized in the two **HA-1**-incompatible combinations. In one of these combinations, which was sex mismatched, all 56 clones analyzed were directed against **HA-1**, demonstrating the dominance of this mHag. In the four **HA-1**-compatible, sex-mismatched combinations, the anti-H-Y response was directed against one immunodominant epitope rather than against multiple Y-chromosome-encoded epitopes. All male specific cytotoxic T lymphocytes (n=15) recognized the same high-performance liquid chromatography-purified peptide fraction presented by T2 cells. Moreover, all cytotoxic T lymphocytes tested (n=6) were specific for the SMCY-derived peptide FIDSYICQV, originally described as being the H-Y epitope recognized in the

context of HLA-A*0201. CONCLUSIONS: Some histocompatibility antigens are recognized in an immunodominant fashion and will therefore be recognized in the majority of mismatched combinations. Only for such antigens, correlations between mismatches and the occurrence of GVHD or graft rejections will be found.

L23 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 11
1999036482. PubMed ID: 9820596. Genomic identification of the minor histocompatibility antigen **HA-1** locus by allele-specific PCR. Wilke M; Pool J; den Haan J M; **Goulmy E.** (Department of Immunohematology and Bloodbank, Leiden University Medical Center, The Netherlands.. ihbsecr@euronet.nl) . Tissue antigens, (1998 Oct) 52 (4) 312-7. Journal code: 0331072. ISSN: 0001-2815. Pub. country: Denmark. Language: English.

AB Graft-versus-host disease (GvHD) can be a major complication of allogeneic bone marrow transplantation even in recipients of HLA genotype-identical transplants. Disparities in minor histocompatibility antigens (mHags) between donor and recipient are a potential risk for the development of GvHD. A mismatch for the mHag **HA-1** can cause GvHD in adult recipients of allogeneic bone marrow from HLA-identical donors. The mHag **HA-1**, first identified by HLA-A*0201-restricted **cytotoxic T cells** (CTLs), was recently chemically characterized as a nonapeptide. On the cDNA level, the **HA-1** locus has two alleles, HA-1H and HA-1R, which differ in two nucleotides, resulting in a single amino acid substitution. Here we report on the genomic structure of the **HA-1** locus. Isolation and sequencing of cosmid DNA encoding the **HA-1** peptide sequence revealed that the **HA-1** alleles are encoded by two exons. Two different primer sets were designed, each consisting of allele-specific primers and a common primer, and both sets containing intronic sequences. We performed genomic DNA typing of three families consisting of 24 HLA-A*0201-positive individuals. The predicted allele-specific products correlated in all cases with the mHag classification by CTLs and by RT-PCR. We demonstrate for the first time the genomic identification of the mHag **HA-1** locus. Prospective genomic typing for the **HA-1** alleles will improve donor selection and identify HLA-A*0201-positive recipients with a high risk for **HA-1**-induced GvHD.

L23 ANSWER 31 OF 37 MEDLINE on STN DUPLICATE 12
97080610. PubMed ID: 8921955. Conservation of minor histocompatibility antigens between human and non-human primates. den Haan J M; Bontrop R E; Pool J; Sherman N; Blokland E; **Engelhard V H**; **Hunt D F**; **Goulmy E.** (Department of Immunohaematology and Bloodbank, Leiden University Hospital, The Netherlands.. haan.j@rulgca.leidenuniv.nl) . European journal of immunology, (1996 Nov) 26 (11) 2680-5. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB It is well accepted that minor histocompatibility antigens (mHag) can function as transplantation barriers between HLA-matched individuals. Little is known about the molecular nature and evolutionary conservation of mHag. It is only very recently that the first human mHag were identified. The HLA-A2.1-restricted mHag HA-2 and the HLA-B7-restricted mHag H-Y appeared to be peptides derived from polymorphic self proteins. Here we show that the HLA-A2.1-restricted mHag **HA-1**, HA-2, and the H-Y peptides are conserved between man, chimpanzees and rhesus macaques. Human **cytotoxic T cell** clones specific for the HLA-A2.1-restricted mHag **HA-1**, HA-2, and H-Y recognized HLA-A2.1 gene-transfected chimpanzee and rhesus macaque cells. High-pressure liquid chromatography fractionation of HLA-A2.1-bound peptides isolated from the HLA-A2.1-transfected chimpanzee cells revealed that the chimpanzee **HA-1** and HA-2 co-eluted with the human **HA-1** and HA-2. Subsequent amino acid sequencing showed that the chimpanzee HA-2 peptide is identical to the human HA-2 peptide. Our functional and biochemical results

demonstrate that mHag peptides are conserved for over 35 million years.

L23 ANSWER 32 OF 37 MEDLINE on STN DUPLICATE 13
96133739. PubMed ID: 8532022. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. **Goulmy E**; Schipper R; Pool J; Blokland E; Falkenburg J H; Vossen J; Gratwohl A; Vogelsang G B; van Houwelingen H C; van Rood J J. (Department of Immunohematology and Blood Bank, Leiden University Hospital, The Netherlands.) New England journal of medicine, (1996 Feb 1) 334 (5) 281-5. Journal code: 0255562. ISSN: 0028-4793. Pub. country: United States. Language: English.

AB BACKGROUND. Graft-versus-host disease (GVHD) can be a major complication of allogeneic bone marrow transplantation even when the donor and recipient are siblings and share identical major histocompatibility antigens. The explanation may be a mismatch of minor histocompatibility antigens. We previously characterized five minor histocompatibility antigens, **HA-1**, 2, 3, 4, and 5, that are recognized by T cells in association with the major histocompatibility antigens HLA-A1 an A2. METHODS. We collected peripheral-blood leukocytes from 148 bone marrow recipients and their sibling donors, who were genotypically HLA identical. Fifty pairs were positive for HLA-A1, 117 were positive for HLA-A2, and 19 were positive for both. The pairs were typed with **cytotoxic-T-cell** clones specific for minor histocompatibility antigens **HA-1**, 2, 3, 4, and 5. RESULTS. Mismatches of HA-3 were equally distributed among recipients in whom GVHD developed and those in whom it did not. By contrast, a mismatch of only **HA-1** was significantly correlated with GVHD of grade II or higher (odds ratio, infinity; P = 0.02) in adults. One or more mismatches of **HA-1**, 2, 4, and 5 were also significantly associated with GVHD (odds ratio, infinity; P = 0.006) in adults. These associations were not observed in children. CONCLUSIONS. A mismatch of minor histocompatibility antigen **HA-1** can cause GVHD in adult recipients of allogeneic bone marrow from HLA-identical donors. Prospective **HA-1** typing may improve donor selection and identify recipients who are at high risk for GVHD.

L23 ANSWER 33 OF 37 MEDLINE on STN DUPLICATE 14
97000507. PubMed ID: 8843592. Functional expression of minor histocompatibility antigens on human peripheral blood dendritic cells and epidermal Langerhans cells. van Lochem E; van der Keur M; Mommaas A M; de Gast G C; **Goulmy E.** (Department of Immunohematology and Bloodbank, Leiden University Hospital, The Netherlands.) Transplant immunology, (1996 Jun) 4 (2) 151-7. Journal code: 9309923. ISSN: 0966-3274. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Adequate presentation and cell surface expression of foreign minor histocompatibility antigens (mHag) to allogeneic T cells can lead to graft versus-host disease (GvHD) after HLA matched bone marrow transplantation (BMT). Cells of the dendritic cell (DC) lineage, including epidermal Langerhans cells (LC), are the most potent inducers of primary alloreactive T cell responses in vivo and in vitro. To explore the possible role of peripheral blood DC and of skin derived LC in the induction of alloimmune responses against mHag, we analysed the functional expression of mHag on these professional antigen-presenting cells (APC). To this end, **cytotoxic T cell** (CTL) clones specific for mHag H-Y and **HA-1** to HA-4 were used to demonstrate the presence of these antigens on highly purified DC and LC. Our results demonstrate that, like other cells of the hematopoietic lineage, DC and LC express all the mHag tested for. The functional expression of mHag on these potent APC suggests their involvement in the induction of mHag specific GvH directed T cell responses after allogeneic BMT.

L23 ANSWER 34 OF 37 MEDLINE on STN DUPLICATE 15

95244869. PubMed ID: 7727778. Interindividual conservation of T-cell receptor beta chain variable regions by minor histocompatibility antigen-specific HLA-A*0201-restricted **cytotoxic T-cell** clones. **Goulmy E**; Pool J; van den Elsen P J. (Department of Immunohaematology and Blood Bank, University Hospital Leiden, The Netherlands.) Blood, (1995 May 1) 85 (9) 2478-81. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Minor histocompatibility antigens (mHags) are involved in the induction of graft-versus-host disease (GVHD) after HLA-identical bone marrow transplantation. Previously, we isolated a series of HLA-A*0201-restricted **cytotoxic T-cell** (CTL) clones specific for the same mHag **HA-1** from peripheral blood of three unrelated patients who were suffering from GVHD. We have now analyzed the composition of the T-cell receptor (TCR) V regions of 12 of these mHag **HA-1**-specific HLA-A*0201-restricted CTL clones by DNA sequencing of the alpha and beta chains. Of these 12 clones, derived from three unrelated individuals, five independent TCR alpha V- and beta V-region sequences were established. The TCR alpha chains were composed of varying TCR alpha V and TCR alpha J genes with no obvious similarities in structure in the N regions. However, the TCR beta chains all used the TCR beta V6S9 gene segment, and showed remarkable similarities within the N-D-N regions; ie, three independent beta-chain sequences (originating from donors Ha and Gy) shared a leucine/valine amino acid pair, whereas the other two (originating from donors Ha and Wi) shared a serine/threonine pair, all at positions 99 and 100 of the TCR beta V region. In conclusion, the TCR analysis of **HA-1** mHag-specific CTL clones has shown that the **HA-1** mHag/HLA-A*0201 complex selects for highly similar TCR beta V regions.

L23 ANSWER 35 OF 37 MEDLINE on STN DUPLICATE 16

94154267. PubMed ID: 8111046. Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by **cytotoxic T-cell** clones. van der Harst D; **Goulmy E**; Falkenburg J H; Kooij-Winkelaar Y M; van Luxemburg-Heijs S A; Goselink H M; Brand A. (Department of Immunohematology and Bloodbank, University Medical Center, Leiden, The Netherlands.) Blood, (1994 Feb 15) 83 (4) 1060-6. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Clinical studies indicated an enhanced antileukemic effect of allogeneic bone marrow transplantation (BMT), as compared with autologous BMT. After allogeneic HLA-identical BMT, donor-derived cytotoxic T lymphocytes (CTLs) directed at minor histocompatibility (mH) antigens on the recipients, tissues can be shown. To evaluate the antileukemic reactivity of mH antigen-specific CTLs, we analyzed the expression of mH antigens on circulating lymphocytic and myeloid leukemic cells. We show that the defined mH specificities **HA-1** through HA-5 and H-Y are present on leukemic cells, indicating that mH antigen-specific CTLs are capable of HLA class I-restricted antigen-specific lysis of leukemic cells. Compared with interleukin-2-stimulated normal lymphocytes, leukemic cells of lymphocytic origin are less susceptible to T-cell-mediated cytotoxicity by the HA-2 mH antigen-specific CTL and the anti-HLA-A2 CTL clone. A possible explanation for this phenomenon is impaired expression of the LFA-1 adhesion molecule. Our study suggests that mH antigen-specific HLA class I-restricted CD8+ CTLs may be involved in the graft-versus-leukemia reactivity after allogeneic BMT.

L23 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 17

94083660. PubMed ID: 8260714. Minor histocompatibility antigens **HA-1**-, -2-, and -4-, and HY-specific **cytotoxic T-cell** clones inhibit human hematopoietic progenitor cell growth by a mechanism that is dependent on direct cell-cell contact. Marijt W A; Veenhof W F; **Goulmy E**; Willemze R; van Rood J J; Falkenburg J H. (Department of Hematology, University Medical Center, Leiden, The Netherlands.) Blood, (1993 Dec 15) 82 (12) 3778-85. Journal code:

7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB HLA-identical bone marrow transplantation (BMT) may be complicated by graft-versus-host disease or graft rejection. Both complications are thought to be initiated by recognition of minor histocompatibility (mH) antigens by HLA-restricted mH-antigen-specific T lymphocytes. Using HLA-A2-restricted mH antigens **HA-1**-, -2-, and -4-, and HY-specific cytotoxic T lymphocyte (CTL) clones, we studied the recognition by these CTL clones of interleukin-2 (IL-2)-stimulated T cells (IL-2 blasts), BM mononuclear cells (BMMNCs), and hematopoietic progenitor cells (HPCs). We showed that, when IL-2 blasts from the BM donors who were investigated were recognized by the **HA-1**-, -2-, and -4-, and HY-specific CTL clones, their BMMNCs and HPCs were recognized as well by these CTL clones, resulting in antigen-specific growth inhibition of erythrocyte burst-forming units (BFU-E), colony-forming units-granulocyte (CFU-G), and CFU-macrophage (CFU-M). the HA-2-specific CTL clone, however, inhibited BFU-E and CFU-G growth from four donors to a lesser extent than from two other donors. We further investigated whether inhibitory cytokines released into the culture medium by the antigen-specific stimulated CTLs or by stimulated BMMNCs were responsible for suppression of HPC growth or whether this effect was caused by direct cell-cell contact between CTLs and HPCs. HPC growth inhibition was only observed after preincubation of BMMNCs and CTLs together for 4 hours before plating the cells in semisolid HPC culture medium. When no cell-cell contact was permitted before plating, neither antigen-stimulated CTL nor antigen-nonstimulated CTLs provoked HPC growth inhibition. Culturing BMMNCs in the presence of supernatants harvested after incubation of BMMNCs and CTL clones together for 4 or 72 hours did also not result in HPC growth inhibition. Both suppression of HPC growth and lysis of IL-2 blasts and BMMNCs in the 51Cr-release assay appeared to be dependent on direct cell-cell contact between target cells and CTLs and were not caused by the release of inhibitory cytokines into the culture medium by antigen-specific stimulated CTLs or by stimulated BMMNCs. Our results show that mH-antigen-specific CTLs can inhibit HPC growth by a direct cytolytic effect and may therefore be responsible for BM graft rejection after HLA-identical BMT.

L23 ANSWER 37 OF 37 MEDLINE on STN DUPLICATE 18

93246305. PubMed ID: 8482585. A genetic analysis of human minor histocompatibility antigens demonstrates Mendelian segregation independent of HLA. Schreuder G M; Pool J; Blokland E; van Els C; Bakker A; van Rood J J; **Goulmy E.** (Department of Immunohaematology, University Hospital Leiden, The Netherlands.) Immunogenetics, (1993) 38 (2) 98-105. Journal code: 0420404. ISSN: 0093-7711. Pub. country: United States. Language: English.

AB An analysis of the genetic traits of human minor histocompatibility (mH) antigens is, unlike with inbred mice, rather complicated. Moreover, the fact that mH antigens are recognized in the context of MHC molecules creates an additional complication for reliable segregation analysis. To gain insight into the mode of inheritance of the mH antigens, we relied upon a series of HLA-A2-restricted **cytotoxic T-cell** (CTL) clones specific for four mH antigens. To perform segregation analysis independent of HLA-A2, we transfected HLA-A2-negative cells with the HLA-A2 gene: this results in the cell surface expression of the HLA-A2 gene product and, if present, mH antigen recognition. The mode of inheritance of the HLA-A2-restricted mH antigens **HA-1**, -2, -4, and -5 was analyzed in 25 families whose members either naturally expressed HLA-A2 or were experimentally rendered HLA-A2-positive. Analysis of distribution of the mH antigens in the parent population among the mating types, together with their inheritance patterns in the families, demonstrated that the four mH antigens behaved as Mendelian traits, whereby each can be considered a product of a gene with two alleles, one expressing and one not expressing the detected specificity. We also showed that the loci encoding the **HA-1** and HA-2 antigens are not closely linked to HLA (lod scores Z (0 = 0.05) <-4.0). Some indication was obtained that the HA-4- and

HA-5-encoding loci may be closely linked to HLA. While we are aware of the limited results of this nonetheless comprehensive study, we feel the similarity in immunogenetic traits between human and mouse mH antigens is at least striking.

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